SPRUCE-SEEDBED INTERACTIONS: IS THERE FACILITATION OF RECRUITMENT IN THE BOREAL FOREST-ALPINE TUNDRA ECOTONE? (MEALY MOUNTAINS, LABRADOR, CANADA)

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Spruce-seedbed interactions: is there facilitation of recruitment in the boreal forest-alpine tundra ecotone? (Mealy Mountains, Labrador, Canada)

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Abstract

As climates warm, black spruce (*Picea mariana*) is expected to expand its range into alpine tundra. Ecological factors such as seedbed and predation may influence recruitment on regional scales. In the boreal forest, seedbed-seedling competition dominates but in the climatically-stressed Mealy Mountains forest-tundra ecotone (Labrador, Canada) the Stress Gradient Hypothesis (SGH) predicts facilitation as the dominant plant interaction. We investigated mechanisms of seedbed facilitation (temperature, water, nutrients, physical structure) in *Pleurozium schreberi, Cladina spp.*, and mineral soil, and examined links between predation and seedbed. *Pleurozium* seedbeds had strong positive effects on seedling growth (30.9%) and survival (55%). Conversely, seedlings recruited poorly on mineral soil, with lowest growth (20.5%) and survival (26%). Seed emergence was highest on *Pleurozium* (6.3%), and lowest on *Cladina* (0.3%). The physical structure of *Pleurozium* likely protects seedlings from exposure, temperatures extremes and predators. As climates warm and seed availability increases, *Pleurozium* may facilitate black spruce expansion.

Keywords: facilitation, *Picea mariana,* seedling survival, seedbed, *Pleurozium schreberi,* mineral soil, alpine treeline, Labrador

Contents

Acknowledgements..... v

1.0 Introduction and overview..... 1

1.1 Seedbed facilitation in the forest-tundra ecotone..... 1

1.2 Study site: Mealy Mountains, Labrador, Canada..... 8

1.3 Thesis rationale and objectives..... 9

Co-authorship statement..... 11

Literature cited..... 12

2.0 Mechanisms of seedbed facilitation of black spruce (*Picea mariana*) recruitment at the treeline..... 17

Abstract..... 17

2.1 Introduction..... 19

2.2 Methods..... 25

2.3 Results..... 33

2.4 Discussion..... 44

Literature cited..... 57

3.0 Conclusions..... 64

Literature cited..... 67

Appendix 1..... 69

Appendix 2..... 71

Appendix 3..... 74

Appendix 4..... 75

Appendix 5..... 77

Appendix 6..... 80

Literature cited..... 82

List of figures and tables

Table 1.1.1: Positive and negative seedbed interactions..... 5

Table 2.3.1: Incidence of black spruce seed emergence 2008..... 35

Table 2.3.2: Odds of seed predation 2008 38

Table 2.3.3: Odds of seedling slug herbivory 2007..... 39

Table 2.3.4: Odds of seedling slug herbivory 2007-2008..... 40

Table 2.3.5: Odds of overwinter mortality 2007-2008..... 43

Table 2.3.6: Odds of root inhibition 2008..... 44

Table A2.1: Proportion of variance explained in nutrient/seedbed PCA 72

Table A5.1: Incidence of black and white spruce seed emergence 2008..... 78

Figure 1.2.1: Map of study area, Mealy Mountains, Labrador..... 26

Figure 1.2.2: Experimental plot study design..... 28

Figure 2.3.1a&b: Temperature and moisture on experimental seedbeds..... 34

Figure 2.3.2a&b: Seedling weighted percent height increase 2007-2008 36

Figure 2.3.3: Black spruce seedling survivorship curves 41

Figure 2.3.4: Proportion of mortality for black spruce in 2007..... 42

Figure 2.3.5: Proportion of mortality for black spruce in 2007-2008..... 42

Figure A2.1: Nutrient/seedbed PCA biplot..... 73

Figure A3.1: Seedling weighted percent height increase (non-reference)..... 74

Figure A4.1: *Deroceras laeve* specimen..... 75

Figure A5.1: Percentage of black spruce (bS) and white spruce (wS) emergence... 78

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I am the Lorax, I speak for the trees, I speak for the trees for the trees have no tongues.

T. Geisel

1.0 Introduction and overview

1.1 Seedbed facilitation in the forest-tundra ecotone

The altitudinal treeline is one of the most extensively studied boundary zones, marking the area of transition between boreal forest and alpine tundra (Körner 1998). This transition zone is maintained and defined by a number of interacting factors, both biotic and environmental (Beaudoin and Head 2004). While climate is considered to be the major driver of altitudinal treeline position across large spatial scales (eg. Holtmeier and Broll 2005), it is important to consider other environmental and biotic influences operating at localized scales such as soil properties (temperature, moisture and nutrients), microtopography, animal activity and interactions among plant species (Butler et al. 2004, Holtmeier and Broll 2005, Holtmeier and Broll 2007, Malanson et al. 2007). Because altitudinal treeline studies often show less advance than expected considering the magnitude of local climate change (Holtmeier and Broll 2007), this suggests that interacting biotic and abiotic factors likely play a role in the spatial variability of treeline movement.

Seedbed, the substrate groundcover at the site of seedfall, forms the dominant interaction matrix for conifer seedling recruitment during the critical early life history stages of emergence and establishment (Duchesneau and Morin 1999, LePage et al. 2000). In the boreal forest, the effects of seedbed on black spruce (*Picea mariana* (Mill) B.S.P.) seedling survival and recruitment have been extensively studied (eg. Prévost

1997, Charron and Greene 2002, Houle and Fillion 2003, Hébert et al. 2006). However, the importance of seedling-seedbed interactions have never been evaluated in the transition area between the boreal forest and the alpine tundra, even though microsite type can be critical for seedling recruitment (Malanson et al. 2007, Germino et al. 2002, Maher and Germino 2006).

Mosses and lichens form a structured substrate cover that can influence soil temperature, moisture regime, and available nutrients, thus altering the physical and chemical conditions of the microhabitat compared with those of non-vegetated substrate (eg. Bonan and Shugart 1989, Deluca et al. 2002, Stark and Hyvarinen 2003). Scarification, or removal of the moss or lichen layer and exposure of the underlying mineral soil, is a common technique in the boreal forest for increasing black spruce seedling recruitment (see Hébert et al. 2006 for examples). The mechanisms behind soil scarification in promoting conifer recruitment are decreased direct competition for water, nutrients and light, and a decline in indirect competition (allelopathy and ectomycorrhizal interference) (Hébert et al. 2006). Thus, it is competition that is inferred to be the dominant interaction between black spruce seedlings and vegetative seedbeds in the boreal forest.

Environmental conditions also play a role in structuring interactions among plants. According to the Stress Gradient Hypothesis (SGH) originally proposed by Bertness and Callaway (1994), the benefits of growing in proximity to neighbouring vegetation can outweigh the disadvantages of potential competition and

physical/chemical inhibition, particularly in harsh environments, where facilitative effects outweigh competitive and inhibitory effects in high-stress habitats during the early seedling stage (Lortie and Callaway 2006, Brooker et al. 2008). In the transition zone between boreal and alpine tundra habitats, black spruce seedlings are living at the limits of their biological and physical tolerance range, a high-stress habitat. Most studies describing facilitative interactions in the treeline ecotone focus on interactions between seedlings and tree-layer vegetation, (Germino et al. 2002, Smith et al. 2003, Maher et al. 2006), but several discuss evidence of facilitative interactions between species of similar sizes (i.e., conifer seedlings and herbaceous layer species) (Choler et al. 2001, Maher et al. 2005). None, however, discuss the potential facilitative effects on conifer seedlings generated by the vegetated seedbed. The seedbed forms the most direct contact with the seedlings through all the stages of its early life history (germination, emergence, and establishment). Under conditions of environmental stress present at the treeline, such as low temperatures, limited growing seasons due to extended snow burial, wind and water stress (Stevens and Fox 1991, Malanson et al. 2007, Weiser and Tausz 2007), a vegetated seedbed could act as a facilitator as opposed to an inhibitor of seedling growth and survival through its influence on soil moisture, temperature and nutrient regimes, and a sheltering effect, especially in the first few years of growth.

The distribution of continuous seedbeds differs between the boreal forest and the forest-tundra ecotone. The feathermoss *Pleurozium schreberi* (Brid.) Mitt. is the dominant moss species in much of the boreal forest, forming continuous mats that can account for 60-80% of groundcover in the boreal ecosystem (DeLuca et al. 2002). In the

central and eastern boreal forest, caribou lichen, *Cladina stellaris* (Opiz.) Brodo, dominates and forms continuous mats within the woodlands that occupy the central area of the Canadian boreal forest (Payette et al. 2000, Simard and Payette 2001). In the Mealy Mountains (south-central Labrador, Canada) forest-tundra transition zone, these seedbeds do not form a continuous groundcover. Rather, mosses and lichens grow in interdigitating patchy mats. For example, in four 100-m transects in the Mealy Mountains, moss and lichen coverage averaged 20 and 15% respectively and ranged from 5 - 100% on each square metre surveyed (LHRG PPSA unpublished data 2008). Bare ground, another potential seedbed, can be exposed by bear digs (scarified areas where the vegetation cover has been removed and the mineral soil exposed by foraging black bears (*Ursus americanus*)). Caribou herbivory was not a significant contributor to lichen seedbed removal in the study area. Bare ground coverage averaged approximately 1%, thus representing a relatively rare microhabitat in the Mealy Mountains forest-tundra ecotone (LHRG PPSA unpublished data 2008).

In this study, I examined *Cladina, Pleurozium schreberi*, and exposed mineral soil (simulated scarified bear digs) as potential seedbeds for black spruce establishment. The predicted balance between the negative and positive interactions within different seedbeds was expected to determine how each seedbed would influence black spruce survival and growth (Table 1.1.1). According to the SGH, positive facilitative interactions should outweigh negative interactions in the environmentally stressful treeline ecotone.

Table 1.1.1: Physical and chemical characteristics of three tested black spruce seedbeds, with potential positive and negative influences on seedling success

Seedbed type	**Distribution/habitat**	**Mechanism(s) of positive seedling interaction(s)**	**Mechanism(s) of negative seedling interaction(s)**
Cladina spp.	Common groundcover in boreal, tundra and alpine habitats (Ahti and Oksanen 1990) Establish in open, unshaded or slightly shaded areas (Bonan and Shugart 1989).	Restrict evaporation from soil surface (Bonan and Shugart 1989, Suzuki et al. 2007) Nutrient mobilization through periodic leaching of usnic and perlatolic acids into soil (Stark and Hyvarinen 2003; Stark 2007)	Associated with water-deficient habitats (Hébert et al. 2006) Lowers soil temperature due to low thermal conductivity (Bonan and Shugart 1989) Damage root systems of young seedlings during expansion and contraction associated with changes in water availability (Boudreau, pers.comm.)
Pleurozium schreberi	Common and often dominant groundcover in boreal forest, tundra and bog peatlands (Benscoter and Vitt 2007) Occupies shaded habitat under partial to full canopy cover; cannot survive in exposed conditions (Bonan and Korzuhin 1989, Bisbee et al. 2001).	Potential nutrient mobilization through association with nitrogen-fixing *Nostoc* cyanobacteria (DeLuca et al. 2002, Houle et al. 2006, DeLuca et al. 2007) Maintains soil moisture levels through absorption and retention of water (Bonan and Shugart	Intercepts and sequesters nutrients from precipitation, throughfall, and plant litter (Wardle et al. 1997; Zackrisson et al. 1999; DeLuca et al. 2002) Lowers soil temperature due to low thermal conductivity

		1989)	(Bonan and Shugart 1989)
Simulated bear dig	Disturbance to seedbed and upper soil layers that occur in grizzly bear habitat due to foraging activities for roots and insect larvae (Tardiff and Stanford 1998); Observed result of black bear foraging in the Mealy Mountains	Decreased direct and indirect competition (interception of water by competing species, shading, allelopathy, etc.) (Hébert et al. 2006) Higher soil temperatures (relative to intact seedbed) (Hébert et al. 2006, Cairns et al. 2007) Increased N availability through pedoturbation (Tardiff and Stanford 1998, Cairns et al. 2007) Increased soil penetrability (Butler et al. 2004, Malanson 2007)	Increased soil surface evaporation may lead to desiccation (Hébert et al. 2006) Exposes soil to erosion (Cairns et al. 2007, Malanson et al. 2007)

Pleurozium, Cladina and bear digs differ in their physical structure and their effect on temperature, moisture and nutrient availability, and they may act to either facilitate or inhibit the success of black spruce germination and growth. This study examined whether the pattern of interaction with vegetated seedbeds leans more towards facilitation or competition. Facilitative interactions may play a key role in conifer establishment and the expansion of forests into the alpine tundra by enhancing recruitment; a genetically-diverse treeline cannot advance upslope until the critical early-

life history stages such as seed germination and seedling establishment occur above the current boundary altitude (Smith et. al 2003).

Seedbed can also facilitate or inhibit seedling establishment and growth through its influence on biotic factors such as small herbivore activity (Côté et al. 2005). In the forest-tundra ecotone, herbivores may reduce seedling growth and survival by removal of foliage and trampling (Cairns and Moen 2004, Cairns et al. 2007). Moreover, post-dispersal seed predation and browsing or grazing of seedlings may play an important role in the limitation of altitudinal treeline advance. For example, Castro et al. (1999) demonstrated that post-dispersal seed predators removed up to 96% of seed across all microhabitats at the treeline of a relict alpine *Pinus sylvestris* forest. In the case of seedlings, the negative effects of herbivory on survival may be magnified by environmental stress (Cairns and Moen 2004). Thus, seedbed may be important in either inhibiting or promoting seed predation and seedling herbivory at these early life history stages; this is the case in the boreal forest, where increased invertebrate seedling predation has been associated with recently burned low-vegetation habitats relative to feathermoss and lichen-dominated habitats (Côté et al 2005). In the Mealy Mountains, invertebrate predators such as slugs are known to feed on 1st-year seedlings in the treeline habitat (Munier 2006). However, to date, no studies have examined associations between seedbed and slug predators in the forest-tundra ecotone. In this study, organic slug pesticide was used to examine the effects of slug herbivory on the different seedbeds.

1.2 Study site: Mealy Mountains, Labrador, Canada

This study was conducted in the Mealy Mountains, approximately 20 km southeast of Lake Melville (N 53° 36.6' W158° 49.0'). The site falls within the boundaries of the proposed Mealy Mountains / Akamiuapishku National Park. The experimental plots for this study were established above the discontinuous forest limit, in the forest-tundra transition zone at approximately 600 m asl. The most abundant conifer species present in the area is black spruce (*Picea mariana),* though white spruce (*Picea glauca* (Moench) Voss.), balsam fir (*Abies balsamea* (L.) Mill) and eastern larch (*Larix laricina* (Du Roi) K. Koch) are present at lower abundance (R. Jameson, unpublished data). The groundcover is dominated by the feathermosses *P. schreberi* and *Hylocomium splendens* (Hedw) Br. et Sch. where shade is present, by *Sphagnum* in wetter areas and by caribou lichens *Cladina stellaris* and *C. arbuscula* (Wallr.) Hale & Culb. in open, unshaded areas. The shrub layer is mainly comprised of arctic dwarf birch (*Betula glandulosa* Michx.) and ericaceous species such as bilberry (*Vaccinium uliginosum* L.) and black crowberry (*Empetrum nigrum* L.) (Cranston 2009). An extensive ground search covering 300 m^2 of forest-tundra habitat near the study area revealed no naturally occurring conifer seedlings (Cranston 2009), although black spruce seedlings have been found at approximately 500 asl (100 m below the study site) (Jameson, unpublished data).

Climate monitoring for the area has been ongoing since 2001, with recording stations at 570, 600 and 995 m asl. Temperatures range from cool summers (13.2°C at

570m asl (July average)) to cold winters (-15.4°C at 570m asl (January average)). Elevation strongly influences temperature, with a lapse rate of approximately -0.7°C per 100 m. Yearly precipitation averages between 2000-3000 mm. The permafrost layer is discontinuous, and annual mean soil temperatures at a depth of 1 m range from -0.1°C at 570 m asl to -0.7°C in the alpine tundra (Jacobs et al. 2007).

In Labrador, local temperatures are expected to warm by 2°C by 2090 which is a smaller climatic shift than other parts of the Canadian north due to the potential cooling effects of the Labrador Current. In terms of the potential for upward tree migration, a shift of 1°C towards a warmer average climate could potentially translate to a 140 m migration upslope (based on purely climatic variables) (Jacobs et al. 2007). However, treeline change is also mitigated by local biotic and environmental effects.

1.3 Thesis rationale and objectives

Soil temperature, moisture and nutrients may constrain treeline advance (Smith et al. 2003, Butler et al. 2004, Holtmeier and Broll 2005, Malanson et al. 2007), but little effort has been made to link critical soil resources to seedbed in the transition treeline habitat, despite the influence seedbed exerts on soil and microhabitat properties. While much of the research has been focussed on critical seedling microsite availability (Malanson et al. 2007, Germino et al. 2002, Maher and Germino 2006), none have examined how moss and lichen seedbeds act to facilitate or inhibit black spruce seedling establishment during the crucial first stage of alpine colonization. Further, only one

study has been made linking invertebrate predators to seedbeds in the boreal forest (Côté et al. 2005); Munier (2006) suggested native slugs graze from a light to moderate extent on black spruce seedlings in the Mealy Mountains forest-tundra ecotone, but did not examine links between slug herbivory and seedbed.

Microsite limitation may be an important environmental factor limiting tree expansion into the alpine region (Malanson et al. 2007, Germino et al. 2002, Maher et al. 2006), but to date, no studies have examined which seedbeds form optimal recruitment microsites in the forest-tundra ecotone. Understanding the role of seedbeds in potential treeline advance contributes to our understanding of how biological interactions will influence tree species expansion as a consequence of climate warming. The altitudinal advance of trees reduces genetic diversity, biodiversity and areal extent of alpine habitats; it can also alter both the water and nutrient budgets in the overall ecosystem (Munier 2006, Malanson et al. 2007).

This study compared three seedbeds, *Cladina*, *Pleurozium* and simulated scarified bear digs, in order to compare black spruce recruitment (germination, growth and survival) and herbivory on each seedbed type. The purpose was to determine whether seedbed-spruce facilitation was occurring, and if so, to infer possible causative mechanisms of seedbed-seedling facilitation (nutrients, water, temperature, physical structure). Black spruce was chosen as an experimental species because it is a) the dominant conifer in the Mealy Mountains forest-tundra ecotone and in other subarctic treelines (Gamache and Payette 2004), b) the largest seed producer in the Mealy

Mountains and c) has naturally occurring seedlings at lower altitudes in the closed-canopy forest (Jameson, unpublished).

Specifically, I examined:

- Differences in abiotic factors (soil temperature, water availability and nutrient supply rate) among *Pleurozium, Cladina* and bare soil seedbed
- Differences in black spruce seed germination among seedbed treatments (*Cladina*, *Pleurozium* and simulated scarified bear digs) to determine the their effect on emergence success
- Differences in black spruce seedling growth, herbivory, and survivorship over a period of two growing seasons among seedbeds and pesticide treatments to determine their effects on recruitment

Co-authorship statement

The manuscript for this thesis was co-authored by Dr. Luise Hermanutz and Dr. Paul Marino of Memorial University of Newfoundland. I was the principal contributor for all aspects including the initial proposal, experimental design and implementation of field methods, data analysis and the preparation of this manuscript.

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2.0 Mechanisms of seedbed facilitation of black spruce (*Picea mariana*) recruitment at the treeline

Abstract

As climate warms, conifers are expected to expand their ranges into alpine tundra. Ecological factors such as seedbed and post-dispersal seed and seedling predation may negatively affect conifer expansion on regional scales. Seedbed provides microhabitat structure and influences temperature, moisture, and nutrients, thus influencing conifer recruitment. In the boreal forest, seedbed-seedling competition dominates, such that seedbed removal increases black spruce recruitment, but in the climatically-stressed Mealy Mountains forest-tundra ecotone (Labrador, Canada) the Stress Gradient Hypothesis (SGH) predicts that facilitation may be the dominant seedbed interaction. This study investigated the potential mechanisms of seedbed facilitation (temperature, water, nutrients, physical structure) in three conifer seedbeds (feathermoss *Pleurozium schreberi,* lichen *Cladina spp.*, and bare ground as simulated bear digs) and examined links between predation and seedbed. The results of this study demonstrated compelling evidence of facilitation between *Pleurozium* and black spruce. *Pleurozium* seedbed had a strong positive effect on seedling growth (30.9%) and survival (55%) and had the lowest overall herbivory, seed predation and overwinter mortality. Conversely, seedlings recruited poorly on bare mineral soil, with lowest overall growth (20.5%) and survival (26%). Seed emergence was low overall (<10% on all treatments), but highest on *Pleurozium* (6.3%), followed by bare ground (4.6%) and *Cladina* (0.3%). Temperature and water availability were similar across all seedbeds, while nutrient availability was

higher on *Pleurozium*. The physical structure of *P. schreberi* likely reduces seedling exposure, and protects from temperatures extremes and predators. As climate warms and seed availability increases, *Pleurozium* may facilitate black spruce recruitment and future treeline expansion.

Keywords: facilitation, *Picea mariana,* seedling survival, seedbed, *Pleurozium schreberi,* mineral soil, alpine treeline, Labrador

2.1 Introduction

The altitudinal treeline transition between boreal forest and alpine tundra is one of the most extensively studied boundary zones (Körner 1998) and is maintained by interacting biological and environmental factors (Beaudoin and Head 2004). Climate may be the primary driver of altitudinal treeline position across landscape and continental scales (eg. Holtmeier and Broll 2005), but important environmental and biotic influences operate at more localized spatial scales, including soil properties (temperature, moisture and nutrients), animal activity and plant interactions (Butler et al. 2004, Holtmeier and Broll 2005, Holtmeier and Broll 2007, Malanson et al. 2007). Altitudinal treelines often show less advance than predicted based on the magnitude of local climate change (Holtmeier and Broll 2007), which suggests that local biotic and abiotic factors, such as microsite characteristics, likely affect the spatial variability of tree recruitment and treeline movement.

In natural habitats the pattern of seedling germination and recruitment has been represented by the 'sieve' model where microsites form a lattice of 'safe' and 'unsafe' locations for germination and growth (Harper 1977, Van der Valk 1981). More recent studies discuss microsite limitation as the primary factor controlling plant recruitment across a variety of habitats (e.g. Crawley 1990, Eriksson and Erhlén 1992, Zobel et al. 2000). Safe sites may facilitate recruitment directly through physical features of the microhabitat, or indirectly, through facilitative biotic interactions. Facilitative interactions may be most important for defining safe sites in stressful environments. In

the forest-tundra ecotone, where multiple controlling factors may limit recruitment (low temperatures, short growing seasons due to snow burial, wind and water stress, seed limitation) (Stevens and Fox 1991, Malanson et al. 2007, Weiser and Tausz 2007), microsite facilitation plays a key role in seedling establishment (Germino et al. 2002, Smith et al. 2003, Malanson et al. 2007). In the forest-tundra ecotone, seedlings that germinate in sub-optimal microhabitats usually die; mortality may be a result, for example, of limited carbon uptake and root establishment, reduced mycorrhizal infection or water stress (Smith et al. 2003).

Seedbed (the groundcover at the site of seedfall) strongly influences conifer seedling recruitment during critical stages such as emergence and early establishment (Duchesneau and Morin 1999, LePage et al. 2000). Moss and lichen seedbeds structure the microsite, influence soil temperature, moisture and nutrients, thus altering the physical and chemical conditions of the microhabitat (eg. Bonan and Shugart 1989, Deluca et al. 2002, Stark and Hyvarinen 2003). Across landscapes and at smaller scales, soil temperature and moisture within seedbed microhabitats may play an important role in treeline dynamics (Holtmeier and Broll 2005, Cairns et al. 2007). In conifer establishment, seedbed suitability is often a function of the chemical and physical characteristics of the substrate (Mallick 2003), and in altitudinal treeline environments low alpine soil temperatures may be one of the primary factors limiting treeline elevation (Smith et al. 2003). Although microsite limitation (Malanson et al. 2007, Germino et al. 2002, Maher and Germino 2006) and soil temperature, moisture and penetrability (Smith et al. 2003, Butler et al. 2004, Holtmeier and Broll 2005, Malanson et al. 2007) have been

discussed as potential impediments of tree expansion into the alpine tundra, little effort has been made to link these limitations to seedbed type.

To date, most studies examining black spruce (*Picea mariana* (Mill) B.S.P.) recruitment and seedbed interactions within forested ecosystems have demonstrated that scarified ground (exposed mineral soil) appears to be an optimal seedbed for black spruce establishment relative to moss or lichen groundcovers (e.g. Prévost 1997, Charron and Greene 2002, Hébert et al. 2006). The mechanisms at work behind black spruce recruitment success on mineral soil are suggested to be reduced competition for water, nutrients and light, and a decline in allelopathy and ectomycorrhizal interference (Hébert et al. 2006). Thus, it can be inferred that competition is the dominant seedbed-seedling interaction in the boreal forest.

However, the forest-tundra ecotone is a climatically stressed habitat for black spruce relative to the boreal forest, suggesting that facilitative biotic interactions among plants may play a more important role in black spruce recruitment. The Stress Gradient Hypothesis (SGH), as proposed by Bertness and Callaway (1994), suggests that the benefits of growing in proximity to neighbouring vegetation outweigh the effects of competition and inhibition in environmentally stressful habitats (Brooker et al. 2008) and that facilitative effects are more important than competition and inhibition during the early seedling stage in high-stress environments (Lortie and Callaway 2006). Facilitative interactions at the treeline ecotone are generally studied at the level of higher-canopy vegetation such as adult trees, (eg. Germino et al. 2002, Smith et al. 2003, Maher et al.

2006) and have also been examined in interactions between species of similar sizes (i.e. seedlings and herbaceous layer species; Choler et al. 2001, Maher et al. 2005). None, however, discuss the potential facilitative effects on conifer seedlings generated by the seedbed in the treeline ecotone. In harsh environments, the seedbed could act as a facilitator of seedling growth and survival through its influences on soil moisture, temperature and nutrients, and its sheltering effect. However, as was shown in forested habitats, the seedbed can also negatively affect seedlings by competition (Hébert et al. 2006), sequestering nutrients (Wardle et al. 1997, Zackrisson et al. 1999, DeLuca et al. 2002), lowering soil temperature beyond thermal tolerance (Bonan and Shugart 1989), and by overgrowing and smothering seedlings (Hornberg et al. 1997). Despite these potential impacts, we expect to see a shift to facilitative seedbed interactions in the more stressful treeline environment, as predicted by the SGH.

Seedbed can also facilitate or inhibit seedling establishment and recruitment through its influence on herbivory and granivory (Côté et al. 2005). Post-dispersal seed predation may act as a biological limitation of recruitment at the treeline (Castro et al. 1999); at the treeline in the Mealy Mountains (Labrador), the native slug *Deroceras laeve* (Müller 1774) is an important seedling herbivore (Munier 2006). In the boreal forest there is greater invertebrate predation of black spruce seedlings in recently burned (low-vegetation) habitats than in feathermoss and lichen-dominated habitats (Côté et al. 2005). Since the impacts of herbivores can be amplified by environmental stress, predators can play a key role in seedling survival and recruitment in the treeline ecotone (Cairns and Moen 2004).

Because Arctic temperatures are expected to warm between 2.5-7°C (relative to 1981-2000) by 2100 (Chapman and Walsh 2007) and temperatures are expected to warm in Labrador (Canada) by 2°C by 2090, upslope tree expansion into alpine tundra habitats has been predicted (Munier 2006; Jacobs et al. 2007). Understanding the relationship between seedbed composition and the establishment and success of tree seedlings at the forest-alpine ecotone will aid in predicting the dynamics of upslope expansion of forest into alpine environments and the subsequent threat to the biodiversity and genetic structure of increasingly isolated and shrinking alpine habitats (Munier 2006, Malanson et al. 2007).

The distribution and survival of conifer seedlings in the forest-tundra ecotone may serve as an effective indicator of the dynamics of treeline expansion as a consequence of warming climates. Relative to adult trees, seedlings have a much lower yearly survival, demonstrate a higher vulnerability to environmental stress, and cannot reproduce asexually through layering after initial establishment (Germino et al. 2002). These characteristics result in a more immediate population response to environmental or climatic change than would be obvious in adult trees. A genetically-diverse (i.e. non-layered) treeline cannot advance into alpine tundra habitat until the critical early-life history stages (i.e. seed germination and seedling establishment) can successfully occur above the current boundary (Smith et. al 2003).

Since facilitation may be an important interaction promoting black spruce recruitment in stressful environments, we examined the potential facilitative effects of

vegetated seedbeds in the climatically stressed alpine treeline ecotone in central Labrador. The seedbeds examined in this study are *Pleurozium schreberi* (Brid.) Mitt., *Cladina stellaris* (Opiz.) Brodo and *C. arbuscula* (Wallr.) Hale & Culb., and exposed mineral soil. *Pleurozium schreberi* is the dominant feathermoss species in the boreal forest, where it forms continuous mats that cover 60-80% of the ground in the boreal forest ecosystem (DeLuca et al. 2002). *Cladina spp.* form continuous mats that dominate the eastern lichen woodlands in the Canadian boreal forest (Payette et al. 2000, Simard and Payette 2001). In the forest-tundra ecotone, in contrast, *Pleurozium* and *Cladina* groundcovers, although common, are non-continuous, overlapping and patchy in distribution. Exposed mineral soil is the optimal black spruce seedbed in the boreal forest; in the forest-tundra ecotone, it is generated by freeze-thaw action and pits dug by foraging black bears (*Ursus americanus).* Exposed soil is a relatively rare microhabitat in the Mealy Mountains compared to moss and lichen seedbeds (LHRG PPSA unpublished data 2008). In this study, exposed mineral soil plots were generated by simulating bear digs, small areas where foraging black bears remove the vegetative cover and expose the soil below.

The objectives of this study were: 1) to determine abiotic factors associated with differences in black spruce germination/seedling success among seedbeds (nutrients, water, temperature), and thus potential mechanisms of facilitation in forest-tundra ecotone seedbeds, 2) to determine whether seedbed facilitation is occurring by comparing black spruce germination, growth, survival across seedbed treatments and 3) to compare seed and seedling predation among seedbeds.

2.2 Methods

Study site and experimental preparation

The Mealy Mountains (Labrador, Canada) study area is located approximately 20 km southeast of Lake Melville (N 53° 36.6' W58° 49.0') and falls within the boundaries of the proposed Mealy Mountains / Akamiuapishku National Park. The experimental plots for this study were established above the discontinuous boundary of open canopy forest in the forest-tundra transition zone at approximately 600 m asl. The dominant conifer is black spruce with white spruce (*Picea glauca* (Moench) Voss.), balsam fir (*Abies balsamea* (L.) Mill) and eastern larch (*Larix laricina* (Du Roi) K. Koch) present at lower abundance (Jameson, unpublished data). The groundcover is locally dominated by feathermosses *Pleurozium schreberi* and *Hylocomium splendens* (Hedw) Br. et Sch. where shade is present, by *Sphagnum* in wetter areas and caribou lichens, *Cladina stellaris* and *C. arbuscula* (Wallr.) Hale & Culb. in open, unshaded areas. The shrub layer is dominated by arctic dwarf birch (*Betula glandulosa* Michx.) and low shrubs such bilberry (*Vaccinium uliginosum* L.) and black crowberry (*Empetrum nigrum* L.) (Cranston 2009). Temperatures range from cool summers (13.2°C at 570m asl, July average) to cold winters (-15.4°C at 570m asl, January average) and yearly precipitation averages between 2000-3000 mm. Elevation influences temperature at a lapse rate of approximately -0.7°C per 100 m. The permafrost layer is discontinuous, and annual mean soil temperatures at a 1 m depth range from -0.1°C at 570 m asl to -0.7°C in the alpine tundra (Jacobs et al. 2007).

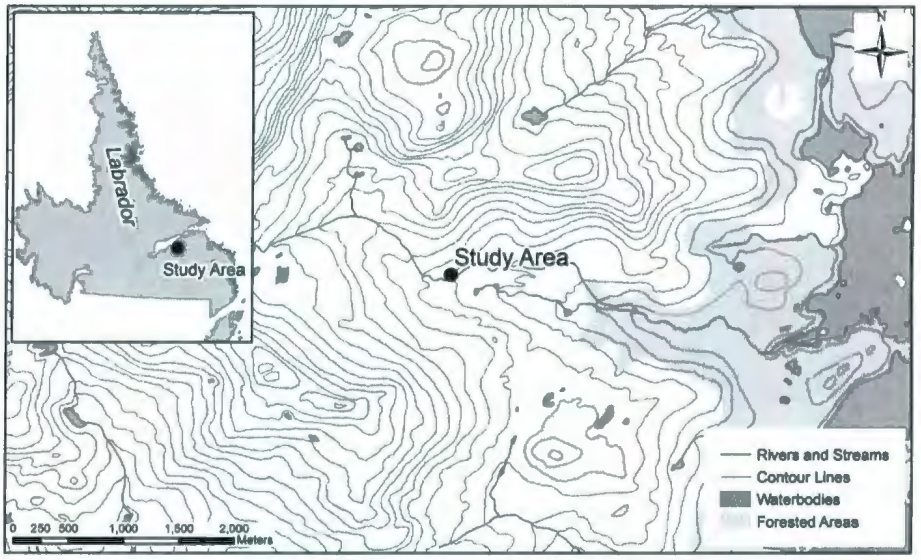

Figure 1.2.1: Map of study area in Mealy Mountains, Labrador; grey area represents approximate position of discontinuous treeline (Map credit Z. Bartlett 2009)

An extensive ground-level search of 300 m^2 of representative habitat near the study area in the forest-tundra ecotone revealed no naturally occurring black spruce seedlings (Cranston 2009) although seedlings were found at lower elevations (~500 m asl) in the forest below the study area (Jameson, unpublished data).

Sixty 25x25 cm experimental plots were established on ground where the *Cladina* or *Pleurozium* cover was greater than 75% (by visual estimation). Because *Pleurozium* is a shade-growing species (Bonan and Shugart 1989) in the Mealy Mountains it generally grows in association with shrubs that provide some canopy cover such as arctic dwarf birch. To minimize the influence of the shrub canopy, the experimental plots were established on the periphery of the dwarf birch stand, or in gaps, such that the experimental plots were never directly shaded. After the locations for the experimental plots were selected and marked, 24 plots (12 of each lichen and moss-dominated) were

haphazardly selected to represent simulated bear digs. In the simulated bear digs, all surface vegetation was removed within a 45 x 45 cm area leaving the bare mineral layer uncovered and disturbed. Because soil desiccation was observed shortly after the removal of the groundcover, extra treatment plots were incorporated into the study to help ensure sufficient seedling survival.

As slug herbivory had been observed in previous studies in the Mealy Mountains forest-tundra ecotone (Munier 2006), slug control was planned as a variable and implemented on half the experimental sites. Thus, plots were established such that each study area was physically separated by a distance of approximately 50 m, reducing probable overlap in slug populations. Herbivory was then controlled at half the sites by means of organic, nutrient-neutral slug pellets; small quantities (less than 5 g) of Safers^{tm} slug and snail bait (0.76% ferric phosphate) were distributed on and around the herbivory-controlled plots every 4 days and following every rainfall during the course of the study. Slope, orientation, vegetation type, soil temperature and moisture were similar in both parts of the study area; the only introduced difference was the presence of slug pesticide.

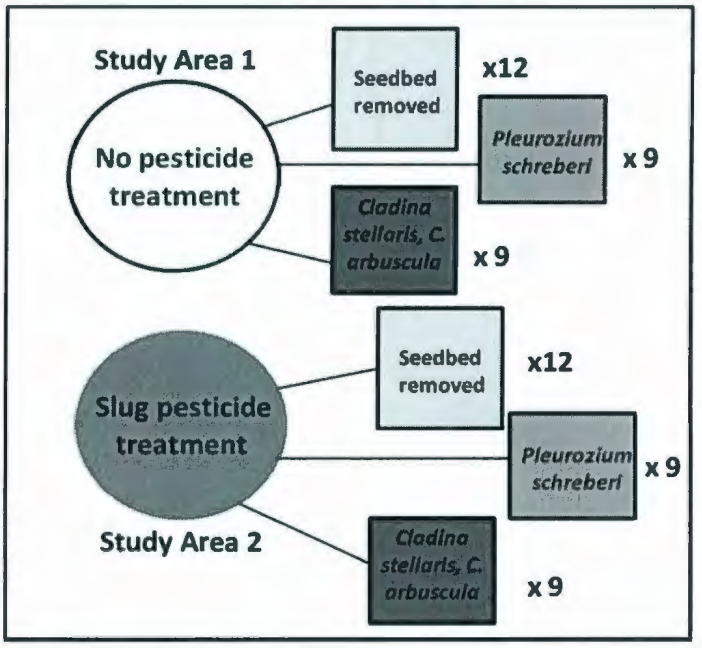

Figure 1.2.2: Experimental seedbed-pesticide plot design in the Mealy Mountains forest-tundra ecotone; study area 1= no pesticide, study area 2 = >5g Safers^{TM} slug and snail bait per study plot

Black spruce seedlings (N=300) were grown from seeds that were collected near Goose Bay (Labrador) within 100 km of the study site by Provincial Forestry Division and grown at the Provincial Nursery in Goose Bay. Seeds were planted in peat pellets without additional fertilizer in June 2007 and were germinated and grown in a greenhouse for 15 days until early July 2007 when they were transported to the Mealy Mountains field site. While in the temperature-controlled greenhouse, the seedlings were grown on raised platforms to prevent contamination by foreign mycorrhizae and were watered daily. Seedlings were transported to the base camp and five were planted in each of the established field plots on July 5th 2007 and monitored for survival, growth and herbivory periodically for two growing seasons. All seedling data included in the

analysis were taken from these experimental plants. Seedling growth, herbivory and survival data were collected on all sixty seedbed plots; abiotic variable data, seed emergence and seed predation data were collected on a subset of these seedling plots.

2.2.1 Seedbed abiotic variable comparison studies

Temperature and moisture

Soil moisture and temperature 8 cm below the seedbed surface were measured to determine whether either factor differed across seedbeds during the height of the growing season. Measurements were collected during July 2008 using a HANNA Instruments probe for temperature (°C) and a Delta-T probe for moisture (% water content). Measurements were not taken within 24 hours of a precipitation event. The data were collected over the course of six (6) 1-2 day intervals in July 2008 on eight (8) plots of each seedbed type.

Seedbed nutrients

Twenty Western AG Plant Root Simulator™ (PRS; Saskatoon, SK) probe sets (8 probes per set) were buried just beneath the seedbed layer in the established treatment plots for 5 weeks (June 24-Aug. 2/08) in order to assess nutrient supply rates and to determine differences in nutrient availability between intact *Pleurozium* and *Cladina* seedbeds. Ten (10) probe sets were planted in each intact seedbed type; simulated bear digs were not included in this study due to limited PRS probe availability. The PRS probe membranes were analysed using colorimetric, inductively-coupled plasma spectroscopy, and flame

emission to determine the absorbed soil nutrient contents by the manufacturer (Western AG Innovations 2007).

2.2.2 Black spruce emergence, growth, predation and mortality

Seed emergence (June 2008-September 2008)

Thirty black spruce seeds (viability >95%) were sown and marked on each of 10 randomly-selected *Cladina* plots, 10 *Pleurozium* plots and 14 bear dig plots from the seedling establishment study on June 23/08 for a total of 1020 seeds. Seeds were monitored daily and emergence was quantified for each seedbed type until July 20/08, then again on Sept 13/08.

Seedling growth (July 2007-September 2008)

Differences in planted black spruce seedling growth (as measured in percent height increase) among groundcover treatments were examined in summer 2007. Calipers (+/-1 mm) were used to measure height on a two-day interval through from July 5 –July 11, with a final measurement taken on September 18. Total seedling height, longest needle length and total number of needles were quantified. In 2008, 3 measurements were taken (June 23, to determine growth and overwinter mortality and on July 18 and September 13). Total seedling height, number of needles and a categorical measure of herbivory damage (see below) were recorded.

Seedling herbivory (July 2007-September 2008)

During the 2007 and 2008 growing seasons, herbivory damage on seedlings was estimated visually when growth measurements were taken. Damage from herbivory was classed categorically based on the percentage of seedling removal: 1= < 25%, 2= 25-50%, 3= 50-75%, 4= >75%, 5= removed. Partial seedling removal was assigned to slugs as their distinctive rasping feeding behaviour removes a portion of the needles over a longer period of time (Moss 2004), while full seedling removal was attributed to vertebrate predators (willow ptarmigan or small rodents). Aside from estimates of herbivory, all other seedling damage and its probable cause (desiccation, drowning, uprooting, etc.) was recorded.

Seed predation (June 2008-July 2008)

Seed predation across the different seedbed and slug control treatments was examined in 2008. Seed predation was examined using seed cards onto which 10 black spruce seeds were lightly glued using 3M™ repositionable spray adhesive to 5×5 cm medium-grade sandpaper squares (Marino et al. 2005). The sandpaper was used for cryptic concealment among the surrounding ground cover, and the light-bond adhesive ensured that seeds could be removed by predators but would not be carried away by the wind. Seed predation was examined in eight randomly selected plots from each seedbed type, for a total of 24 plots and the same plots were used for each experiment repetition. The seed cards were secured to the ground with 2'' roofing nails and were collected and replaced on a 5-day interval, for a total of four replacements. Every disturbed card (i.e., 1+ seed removed) was counted as a predation event since the likelihood of individual seed

removal increased if a predator encountered the card. The data were then pooled for analysis.

Seedling mortality (June 2008-September 2008)

During the 2008 field season (in June and September) all dead planted seedlings were removed from the peat pellet and checked for likely causes of mortality such as herbivory and poor root development. Needle height, total seedling height, and length of primary/secondary roots were recorded in order to determine any visible causes of mortality such as herbivory. Root inhibition was determined to be the cause of mortality if the primary and secondary root systems appeared underdeveloped compared to a healthy seedling. Desiccation was not assigned or analysed as a direct cause of mortality because it could not be attributed with certainty due to the time period between monitoring dates.

2.2.3 Data analyses

Data analyses were carried out using SAS v9.1, Minitab v13 and R 2.6.0. Temperature and moisture were analysed using general linear models (ANOVAs) where temperature and water availability were the response variables, and seedbed and date were the explanatory variables. Nutrient supply totals were analysed using a principal components analysis (PCA) after processing the data by Euclidean normalization (Wen et al. 2007). A Poisson regression model was used for seed emergence, with count of emerged seeds

as the response variable, and seedbed as the explanatory variable. Seedling growth was analysed using a weighted-means ANOVA, in which the response variable was the percent height increase from the initial planted height, and explanatory variables were seedbed and pesticide treatment. Percent height increase for each seedling was weighted by plot survivorship so growth on treatments with fewer surviving seedlings did not have an undue weight. Seedling herbivory, seed predation, overwinter mortality and mortality from root inhibition were all analysed using logistic regression models, in which the response variable was the odds of the event (predation, mortality), and explanatory variables were seedbed and pesticide treatment; whereas overwinter mortality had only 1 explanatory variable, seedbed. Simulated bear digs with no pesticide were chosen as the reference treatment for all logistic regression models (all results for other treatments are expressed as odds ratios *relative* to this treatment) because mineral soil is considered to be the optimal seedbed in the boreal forest (e.g. Prévost 1997, Charron and Greene 2002, Hébert et al. 2006) and thus any significant differences of event likelihood on the other treatments can be attributed to the presence of an intact seedbed and/or slug pesticide. For a more detailed explanation of logistic regression models, see Appendix 1.

2.3 Results

2.3.1 Temperature, moisture and nutrients

Soil temperatures did not differ significantly ($N = 240$, $F = 0.10$, $p = 0.904$) across seedbeds throughout summer 2008. During the July growing season soil temperatures

were lower at the beginning of the sampling period, peaked mid-season, and declined to their lowest values at the end of the sampling period. The average soil temperature was 12.3°C on all treatments through the sampling period (Fig. 2.3.1a). Average percent soil moisture was lowest (23.4%) on bare ground digs, and similar on *Cladina* (26.4%) and *Pleurozium* (26.7%) seedbeds; (Fig.2.3.1b). However, percent soil moisture was not significantly different between treatments at each individual sampling period (12 Jul: N=48, F=0.23, p=0.792; 14 Jul: N=48, F=0.92, p=0.408; 15 Jul: N=48, F=2.74, p=0.075; 16 Jul: N=48, F=1.59, p=0.215; 18 Jul: N=48, F=2.22, p=0.120).

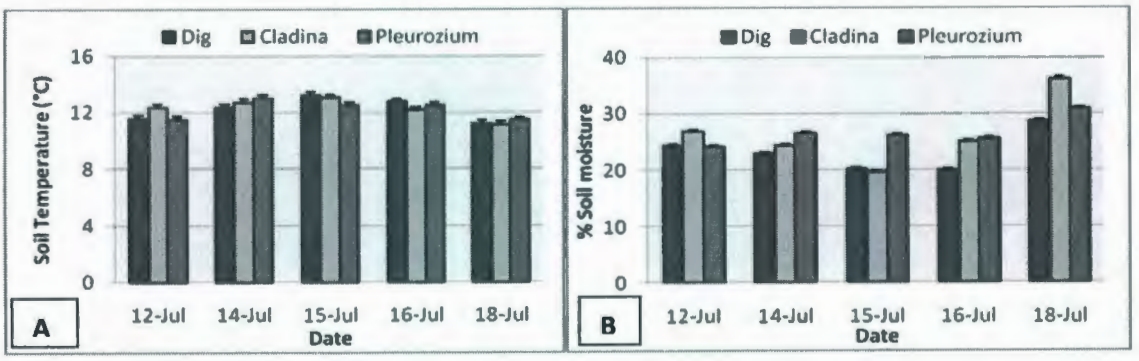

Figure 2.3.1: a) Average soil temperature (°C) across 3 seedbed treatments during the 2008 growing season; b) Average percent soil moisture across 3 seedbed treatments during the 2008 growing season

Analysis of the first two principal component axes explained 71% of the variation in soil nutrient supply rates (Table A2.1, Appendix 2); PCA axis 1 primarily reflected calcium, magnesium and iron concentrations, as indicated by their negative (less than -0.495) component loadings, while PCA axis 2 mainly reflected positive iron and negative sulphur (0.607 and -0.569) loadings. The distribution of *Cladina* and *Pleurozium*

samples on the biplot suggests a stronger positive relation between soil nutrients and *Pleurozium*, and a negative relation to *Cladina,* which suggests the lichen seedbed is more nutrient-deficient for all nutrients except boron when compared to *Pleurozium* (Figure A2.1, Appendix 2). Soil nutrient supply rates were highly variable across *Pleurozium* seedbeds.

2.3.2 Emergence, growth and survival

Emergence of black spruce was very low across all treatments (total 36/1020), but differed significantly across seedbeds over the 2008 growing season (Chi-square=221.19, p=<0.0001). Black spruce emergence was highest on *Pleurozium* (N emergents=19, 6.3%), followed by dig (N emergents=16, 5.3%) and was lowest on *Cladina* (N emergents=1, 0.33%). Seed emergence was earliest on dig treatments (July 12-Sept 13), followed by *Pleurozium* (July 13-Sept 13), with latest emergence on *Cladina* (single seed emerged on July 17).

Table 2.3.1: Incidence ratios (IR) of relative black spruce emergence likelihood by seedbed (2008). Incidence of black spruce emergence on reference treatment Dig =16; ratios >1 = higher emergence likelihood, ratio <1 = lower emergence likelihood

	Incidence Ratio	**IR Confidence Intervals**	
***Cladina* vs Dig**	0.0625	0.0332	0.118
***Pleurozium* vs Dig**	1.188	0.979	1.441

Incidence of black spruce emergence was almost equally likely on digs and *Pleurozium* (OR=1.19), while emergence likelihood was significantly lower on *Cladina* (OR=0.063; Table 2.3.1).

Growth

Average height increase was greatest on *Pleurozium*+ pesticide and *Pleurozium* treatments over the course of two growing seasons (Appendix 3; Figure A3.1). Like the logistic and Poisson regression models, seedling growth across treatments was examined and analysed with respect to growth on the reference treatment, Dig –pesticide. The average percent height increase of black spruce seedlings was greatest on *Pleurozium* + pesticide relative to Dig –pesticide treatment after each of the three measurement periods: after the 2007 growing season (H_{incr}=23.1%), after winter and spring (H_{incr}=25.5%) and after the 2008 growing season (H_{incr}=32.2%; Figure 2.32a).

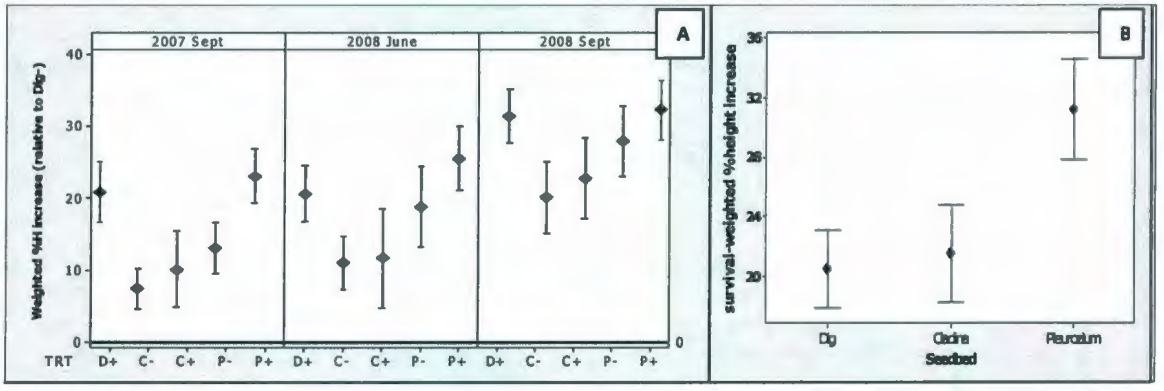

Figure 2.3.2: **A)** Seedling weighted percent height increases (with 95% CI) of seedlings on Dig + (D+), *Cladina* +/- (C+/-) and *Pleurozium* +/- (P+/-) relative to seedlings on Dig- treatments (values greater than y=0 indicate greater relative % height increase); points indicate average percent height increase weighted by survival on each treatment type during each growing period, relative to average percent height increase on dig- during each period and **B)** Seedling weighted percent height increases (with 95% CI) pooled across Dig +/- (D+/-), *Cladina* +/- (C+/-) and *Pleurozium* +/- (P+/-) pesticide treatments after 2 growing seasons (Sept 2008)

In Sept 2007, seedling percent height increase was significantly different across seedbed (N=206, F=6.27, p=0.002) and pesticide treatment (N=206, F=13.16, p=0.0001); average percent height increase (relative to Dig-, the reference treatment) was highest on *Pleurozium*+ (23.1%), followed by Dig+ (20.9%), *Pleurozium*- (13.1%), *Cladina*+ (10.2%), and *Cladina*- (7.5%). In June 2008, seedling height increase was significantly different across seedbed (N=135, F=9.89, p=>0.000), but non-significant between pesticide treatments (N=135, F=269, p=0.103), which was expected since pesticide was not added to the treatment plots between August 2007 and June 2008 (i.e., overwinter); average percent height increase (relative to Dig-, the reference treatment) was highest on *Pleurozium*+ (25.5%), followed by Dig+ (20.6%), *Pleurozium*- (18.8%), *Cladina*+ (11.7%), and *Cladina*- (11.1%). In Sept 2008, after 2 full growing seasons, mean seedling height across all treatments was 33 mm (+/- 1.31 mm). Seedling height increase was significantly different across seedbed (N=102, F=8.13, p=0.001), but was not significantly different across pesticide treatment (N=102, F=2.69, p=0.104). The average percent height increase (relative to Dig-, the reference treatment) was highest on *Pleurozium*+ (32.2%), followed by Dig+ (31.4%), *Pleurozium*- (27.9%), *Cladina*+ (22.7%), and *Cladina*- (20.1%). During all measuring periods, the order of seedling height increase by treatment was the same (highest on Pleurozium+, Dig+, Pleurozium-, Cladina+, Cladina-), with seedlings planted on Dig – pesticide (reference treatment, y=0) experiencing the lowest growth since all lower confidence intervals fall above y=0 (Figure 2.3.2a).

After the first growing season, pesticides had no effect on seedling growth. When measurements for Sept 2008 were pooled across seedbeds, survival-weighted height increase is significantly higher on *Pleurozium* (30.9%) than on *Cladina* (21.7%) or digs (20.5%; Figure 2.3.2b).

Seed predation

During the 2008 growing season, the odds of seed predation were significantly different across seedbeds (Chi-square=31.62, p=<0.0001) and pesticide treatments (Chi-square=17.01, p=<0.0001) with no significant interaction effects (Chi-square=2.14, p=0.3438). The probability of a seed predation event was highest on Dig + pesticide (83%), followed by *Cladina* + pesticide (75%), *Cladina* – pesticide and Dig – pesticide (69%), *Pleurozium* + pesticide (62%) and *Pleurozium* – pesticide (50%).

Table 2.3.2: Odds ratios of relative seed predation likelihood by seedbed and pesticide treatment (2008). Odds of seed predation occurring on reference treatment Dig- =2.2; ratios >1 = higher predation likelihood, ratio <1 = lower predation likelihood

	Odds ratios	**OR Confidence Intervals**	
***Cladina* - vs Dig -**	1	0.688	1.453
***Pleurozium* - vs Dig -**	0.45	0.317	0.652
+ vs -	2.27	1.362	3.791
***Cladina* + vs *Cladina* -**	1.36	0.411	4.529
***Pleurozium* + vs *Pleurozium* -**	1.75	1.049	5.749

During the 2008 growth season seed predation was more likely to occur on pesticide-treated plots than those without pesticide (OR=2.27, less likely to occur on *Pleurozium* plots (OR=0.45) and equally likely to occur on *Cladina* (OR=1) relative to

Digs (odds=2.2; Table 2.3.2). Black spruce seeds were significantly less vulnerable to predation on *Pleurozium* plots relative to *Cladina* and digs.

Seedling herbivory by slugs

While only four slugs were trapped during the 2008 growing season (Appendix 4), the majority (>90%) of seedling herbivory was attributed to slugs. Therefore the analyses were designed to evaluate differences in slug herbivory across treatments. The remaining proportion of herbivory was attributed willow ptarmigan. In circumstances where willow ptarmigan were identified as the herbivore responsible, seedlings were completely removed, peat pellets were uprooted, and ptarmigan scat was found on the plots. During the 2007 growing season, herbivory mortality was significantly different across seedbed (Chi-square= 12.31, p=0.0021). Pesticide treatments (Chi-square= 1.21, p=0.2707) and interaction effects (Chi-square=0.67, p=0.7158) were non-significant; seedling herbivory mortality was highest on Dig+ pesticide (35%), followed by Dig – pesticide (31.7%), *Pleurozium* + pesticide (20%), *Cladina* + pesticide (17.8%), *Cladina* – pesticide (15.6%) and was lowest on *Pleurozium* – pesticide (11.1%).

Table 2.3.3: Odds ratios of relative seedling slug herbivory likelihood by seedbed and pesticide treatment (2007). Odds of herbivory occurring on reference treatment Dig- =0.462; ratios >1 = higher predation likelihood, ratio <1 = lower predation likelihood

	Odds ratios	**OR Confidence Intervals**	
***Cladina* - vs Dig -**	0.4001	0.1514	1.057
***Pleurozium* - vs Dig -**	0.2675	0.09078	0.7884
+ vs -	1.166	0.5451	2.492
***Cladina* + vs *Cladina* -**	1.156	0.1405	9.504
***Pleurozium* + vs *Pleurozium* -**	2.023	0.2314	17.69

During the 2007 growing season, slug herbivory was almost equally likely to occur on pesticide-treated plots than those without pesticide (OR=1.166) and slug herbivory was less likely to occur on *Pleurozium* (OR=0.27) and *Cladina* plots (OR=0.4) relative to Digs (odds=0.46; Table 2.3.3).

Over both 2007-2008 growing seasons, slug herbivory was significantly different across seedbed (Chi-square=15.51, p=0.0004) with no significant differences across pesticide treatments (Chi-square=0.09, p=0.7655) or interaction effects (Chi-square=0.73, p=0.6956). Overall 2-year seedling herbivory was highest on Dig- pesticide (40.0%), followed by Dig + pesticide (35.0%), *Pleurozium* + pesticide (20%), *Cladina* + pesticide (17.8%), and was lowest on *Cladina* – pesticide (15.6%) and *Pleurozium* – pesticide (15.6%).

Table 2.3.4: Odds ratios of relative seedling slug herbivory likelihood by seedbed and pesticide treatment (2007-2008). Odds of herbivory occurring on reference treatment Dig- =0.667; ratios >1 = higher predation likelihood, ratio <1 = lower predation likelihood

	Odds ratios	**OR Confidence Intervals**	
***Cladina* - vs Dig -**	0.277	0.107	0.722
***Pleurozium* - vs Dig -**	0.273	0.105	0.713
+ vs -	0.808	0.385	1.693
***Cladina* + vs *Cladina* -**	1.172	0.147	9.327
***Pleurozium* + vs *Pleurozium* -**	1.373	0.175	10.757

Over the course of 2 growing seasons (2007-2008) herbivory was almost equally likely to occur on pesticide-treated plots than those without pesticide (OR=0.81) and herbivory was approximately equally probable on *Pleurozium* and *Cladina* plots but

significantly less likely to occur on these plots relative to Digs (*Pleurozium* OR=0.27, *Cladina* OR=0.28) relative to Digs (odds=0.58; Table 2.3.4).

Seedling survival

Of the three hundred (300) seedlings initially planted across all six treatment types, 206 seedlings (68.7%) survived the 2007 growing season, while 118 (39.3% of initially planted seedlings) survived through the 2008 growing season (Figure 2.3.3). The proportion of total seedlings surviving was highest on the *Pleurozium* with pesticide treatment (58%) and lowest on the simulated bear dig with no pesticide treatment (22%).

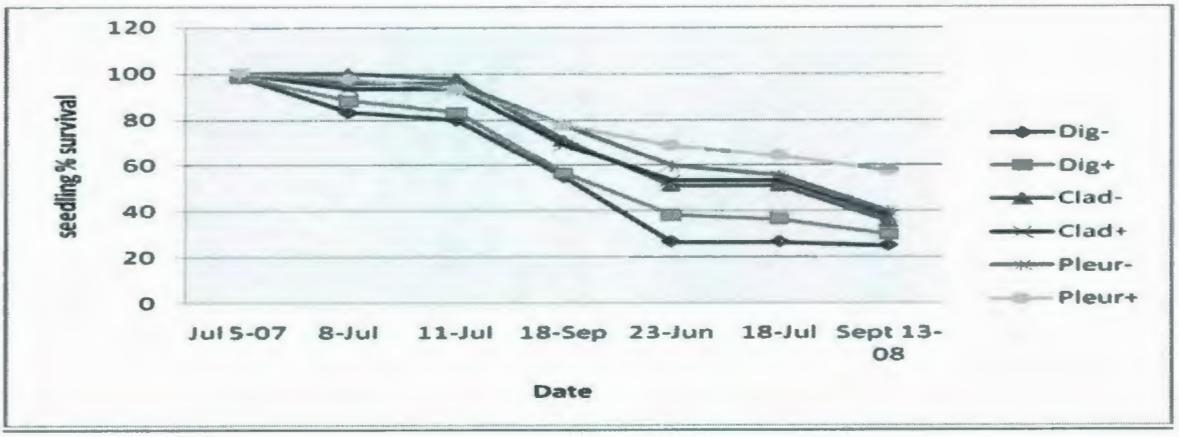

Figure 2.3.3: Survivorship curves showing percent survival of black spruce seedlings on Dig+/-, *Cladina* +/- and *Pleurozium* +/- treatments (2007-2008)

Factors causing mortality in the first growing season included herbivory (84%), drowning (5.3%) and unknown causes (10.6%; Figure 2.3.4). New second-year mortality factors included overwinter mortality (38.1%), desiccation (29.7%), and overgrowth by moss (1.7%; Figure 2.3.5).

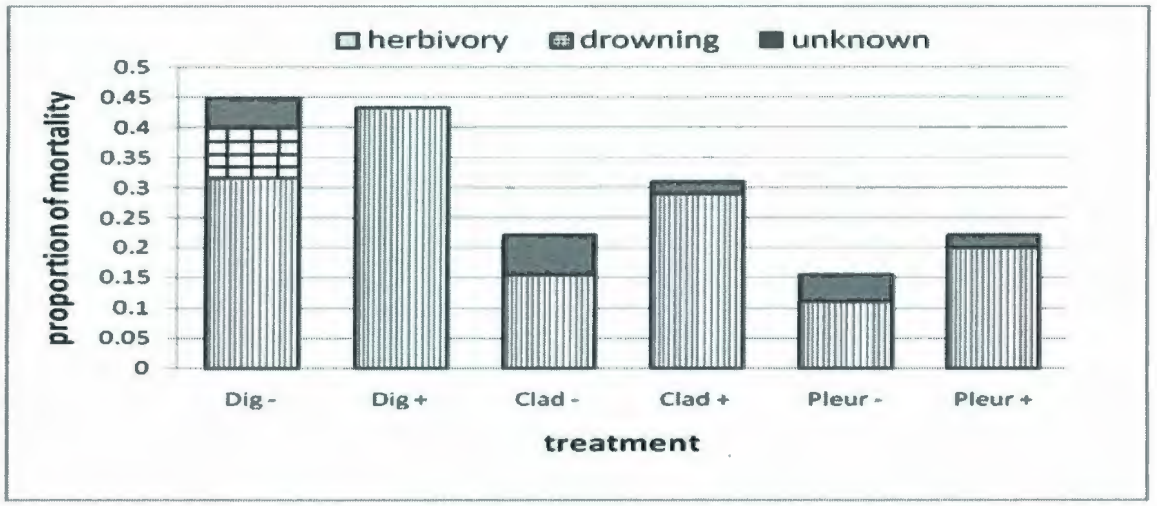

Figure 2.3.4: Total percent mortality and proportion of contributing mortality factors for black spruce seedlings across all treatments (2007)

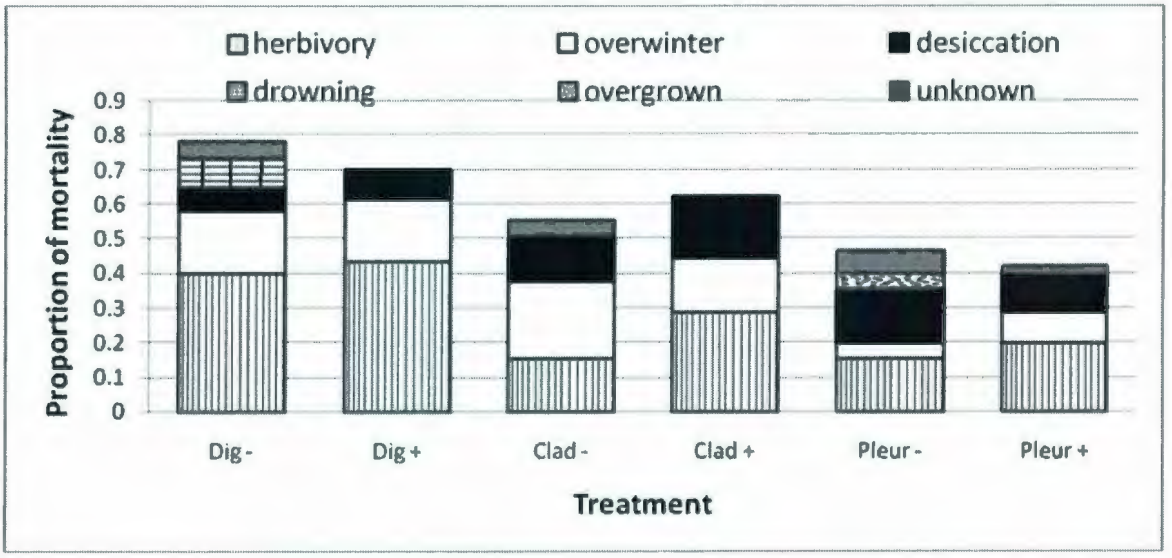

Figure 2.3.5: Total percent mortality and proportion of contributing mortality factors for black spruce seedlings across all treatments (2007-2008)

While slug herbivory was the dominant cause of mortality in the first growing season (84%) (Table 2.3.3 and 2.3.4 for analyses), overwinter mortality was the primary cause of new mortality in 2008 (38.1%). Overwinter mortality was significantly different

across seedbeds (Chi-square=8.07, p=0.0176) as it was highest on *Cladina* (18.9%), followed by Digs (18.3%), and was lowest on *Pleurozium* (6.7%).

Table 2.3.5: Odds ratios of relative seedling overwinter survival likelihood by seedbed, 2007-2008. Odds of overwinter mortality occurring on reference treatment Dig =0.225; ratios >1 = higher predation likelihood, ratio <1 = lower predation likelihood

	Odds ratios	**OR Confidence Intervals**	
***Cladina* vs Dig**	1.027	0.509	2.072
***Pleurozium* vs Dig**	0.313	0.121	0.812

While the odds of overwinter mortality are approximately equal on Digs (odds=0.225) and *Cladina* (OR=1.027) they are significantly lower on *Pleurozium* (OR=0.313; Table 2.3.5).

Root inhibition

Root inhibition through 2007-2008, attributed to any dead seedling with an underdeveloped root system compared to healthy seedlings, was significantly different across seedbed (Chi-square=10.46, p =0.0054) and pesticide treatments (Chi-square= 6.72, p=0.0095) but there were no significant interactions (Chi-square =0.26, p=0.8792). Root inhibition was highest on Dig+ pesticide (43.3%), followed by *Cladina* + pesticide (28.8%), Dig - pesticide (28.3%), *Pleurozium* + pesticide (22.2%), *Cladina* - pesticide and *Pleurozium* – pesticide (13.8%).

Table 2.3.6: Odds ratios of relative seedling root inhibition likelihood by seedbed and pesticide treatment (2008). Odds of root inhibition occurring on reference treatment Dig- =0.4; ratios >1 = higher predation likelihood, ratio <1 = lower predation likelihood

	Odds ratios	OR Confidence Intervals	
***Cladina* - vs Dig -**	0.4	0.139974	1.087194
***Pleurozium* - vs Dig -**	0.4	0.139974	1.087194
+ vs -	1.93	0.90529	4.128029
***Cladina* + vs *Cladina* -**	2.61	0.401318	20.77811
***Pleurozium* + vs *Pleurozium* -**	1.84	0.225305	15.09404

Root inhibition was much less likely to occur on *Pleurozium* (OR=0.4) and *Cladina* plots (OR=0.4) relative to Digs (odds=0.4). On all treatments, root inhibition was more likely to occur on pesticide-treated plots than those without pesticide (OR = 1.93; Table 2.3.6).

2.4 Discussion

2.4.1 Mechanisms of seedbed facilitation

The results of this study demonstrated compelling evidence of facilitation between *Pleurozium* and black spruce. Compared to the other substrates tested, black spruce seeds had a higher incidence of emergence on *Pleurozium* seedbeds, greater height increase after 2 growing seasons and were less likely to die from root inhibition relative to digs or *Cladina* seedbeds. Odds of overwinter mortality, seed predation, and first-year and cumulative slug herbivory were also significantly lower on *Pleurozium* treatments relative to digs. Also, there was no clear pattern of improved success on either *Cladina*

vs. digs relative to each other; black spruce emergence was lower on *Cladina* relative to scarified digs but the odds of root inhibition and first-year/cumulative slug herbivory were lower on digs. The results of this study demonstrate a completely different pattern of black spruce recruitment than that established by the majority of studies conducted in the boreal forest, where scarified ground represents the optimal seedbed for black spruce recruitment, and *Pleurozium* and *Cladina* seedbeds are characterized by mainly negative impacts on seedlings (eg. Prévost 1997, Charron and Greene 2002, Hébert et al. 2006). Only one boreal study reported increased seedling growth on *Pleurozium*: Lavoie et al. (2006, 2007) observed greatest yearly incremental increase in black spruce seedlings planted on *Pleurozium* and *Pleurozium*-derived seedbeds in post-logging lowland boreal forests, and poorer growth on bare soil, likely as a result of bare soil flooding and -*Sphagnum* overgrowth (Lavoie et al. 2006, 2007). We suggest that under the environmentally stressful conditions in the treeline ecotone, *Pleurozium* seedbeds enhance black spruce growth, survival and recruitment, shifting the interaction balance from competition to facilitation. We examined several abiotic variables , including temperature, moisture availability, nutrient availability, and structural protection, and discuss how differences in these properties among the three seedbeds may have facilitated or inhibited black spruce recruitment.

Soil temperatures under moss and lichen seedbeds may be colder than, or more highly insulated and warmer than exposed soil, depending on the season (Bonan and Shugart 1989, Colombo et al. 2003, den Herder et al. 2003). In our study, there were no soil temperatures differences among seedbed during the height of the growing season in

early to mid-July 2008. This month had the highest average temperatures and greatest number of degree days relative to any other month for 2008 (Jacobs and Chan, unpublished). Seedbed temperatures may be more variable between substrate types during the fall, when removal of the insulating seedbed layer can cause extremes in soil temperatures, especially during frost periods before snow cover is established (Colombo et al. 2003, den Herder et al. 2003). The thermal control regulated by seedbed insulating properties may facilitate conifer growth and survival because cold soils are associated with photosynthesis and root growth limitations in conifer seedlings (Smith et al. 2003). The odds of root inhibition and overwinter mortality were much higher on digs and *Cladina* relative to *Pleurozium*, suggesting that moss seedbeds protect seedlings from the low temperatures extremes that likely occur on scarified digs during cold periods when insulating snow cover is absent. *Cladina* spp. have high reflectivity of solar radiation (Bonan and Shugart 1989), so lichen seedbeds are colder than the darker *Pleurozium* patches which would absorb more solar radiation, especially in the spring after the snow cover has retreated.

Soil water availability may be the primary factor limiting black spruce seed germination (Power 2005, Lavoie et al. 2006), especially since all seedbed temperatures during the 2008 growing season exceeded the 10°C minimum germination temperature for spruce (Black and Bliss 1980). *Pleurozium* and *Cladina* seedbeds are usually wetter than exposed soils (Bonan and Shugart 1989, Suzuki 2007, but can desiccate during the boreal summer (Hörnberg et al. 1997, Moss 2004). In our study, seeds and seedlings likely did not experience significant moisture deficits in the 2007 or 2008 growing

seasons. While drought years may occur, moist summers have characterized the Mealy Mountains forest-tundra ecotone during the past 8 years of climate monitoring (Jacobs, per. comm.) Black spruce seeds had nearly equal odds of emergence on *Pleurozium* and digs, with very low odds on *Cladina*. Digs are suggested to be optimal germination sites because developing roots are better able to penetrate bare mineral soil (Butler et al. 2004, Malanson et al. 2007). Germination totals were lowest on *Cladina* seedbed which contrasts to Power's (2005) finding of increased black spruce seed emergence in lichen fractures in Terra Nova National Park (Newfoundland). *Cladina* seedbeds are often characterized as water-stressed (Hébert et al. 2006), which could seriously impact emergence, though that does not seem to be the case in this study area. The extremely low emergence on *Cladina* may be a result of the physical structure of this seedbed; while seeds remained on, or close to the surface of the denser *Pleurozium* and dig plots after sowing, they tended to fall deeper into the fractured, labyrinthine lichen mats. It is possible that from this deeper starting point, emerging black spruce may have been unable to penetrate the upper seedbed surface during the growing season. In a companion study, white spruce seeds demonstrated similarly low emergence to black spruce across all seedbeds, and had a greater likelihood of emerging on all seedbeds except *Pleurozium* (Appendix 5). White spruce was not included in the thesis as only emergence data were available.

Seedling desiccation accounted for a relatively small proportion of the 2-year overall mortality (11%), with no deaths due to desiccation occurring in the first growing season. This result again differs from what others have found in the boreal forests, in

which black spruce germination failure and seedling mortality in *Pleurozium* and *Cladina* seedbeds is often attributed to seedbed desiccation (Hörnberg et al. 1997, Moss 2004, Hébert et al. 2006). This suggests that soil moisture differences among seedbed substrates likely had little effect on seedling establishment and/or growth.

Two-year seedling growth and survival was highest on the *Pleurozium* seedbeds, which may be associated with nutrient availability. Moss and lichen groundcovers are often associated with differences in soil nutrients as lichen groundcovers are generally associated with nutrient-poor soils (Bonan and Shugart 1989, Carleton 1990). The *Cladina* plots in this study followed a similar pattern as they were moderately nutrient-deficient when compared to the *Pleurozium* seedbeds. Calcium in particular was in higher supply on *Pleurozium.* This is likely because of litter leachates from dwarf birch growing in close proximity to the moss seedbeds (DeHayes et al. 1999). Calcium is required for cell wall and membrane integrity and in building tolerances to stressful abiotic conditions such as cold and drought (Fink 1991, DeHayes et al. 1999); the increased availability of calcium in the *Pleurozium* seedbed may have contributed to the higher overwinter survival of seedlings on *Pleurozium* as compared to digs and *Cladina.* There was also greater availability of nitrogen and nitrate on *Pleurozium. Nostoc sphaericum,* a nitrogen-fixing cyanobacterium, has been observed living in association with *Pleurozium* in the boreal forests of Scandinavia and Quebec (DeLuca et al. 2002). In the Mealy Mountains, however, this enhanced nitrogen level could not be positively attributed to a cyanobacterial association, but see Appendix 6 for further discussion.

Mycorrhizae were present in all seedbeds in the study area and were not a barrier to seedling establishment (Kernaghan, pers. comm.).

The physical structure of seedbeds and associated vegetation may facilitate seedlings by sheltering them from low temperatures and photoinhibition (photosynthetic reduction caused by overexposure to high-intensity light; Smith et al. 2003). Full sky exposure increases photoinhibition, which reduces photosynthetic carbon gain and growth, and has been linked to high mortality rates in some conifer species. Sheltering plants reduce sky exposure, which alters the temperature regime (warmer night and cooler day temperatures) and increases photosynthetic carbon gain, root growth, mycorrhizal infection and survival (Smith et al. 2003, Maher and Germino 2006). Seedlings planted in digs have no vegetation growing around them to provide shelter. Since the treeline habitat lacks a continuous canopy, the seedlings in digs are almost completely exposed to the sky, relative to those planted in *Pleurozium* and *Cladina* plots which have some direct shelter provided by the seedbed growing up around each seedling; in the case of *Pleurozium*, there is may be indirect shelter from photoinhibition provided by the shrubs which grow in association with the feathermoss. However, this is unlikely since Cranston (2009) demonstrated that dwarf birch have no facilitative effect on seedling growth and survival during the first two growing seasons, and seedlings planted in *Pleurozium* plots were never directly shaded by shrubs. However, associated shrubs may facilitate overwinter and spring survival of seedlings by accumulating a greater insulating snow layer than more exposed seedbeds (Germino et al. 2002). Since seedling growth was greatest, odds of root inhibition lowest and odds of survival highest

on the *Pleurozium* groundcover, this suggests that the physical structure of this seedbed may directly reduce sky exposure and regulate the temperature regime of the microhabitat, facilitating the recruitment of black spruce. However, further research is needed to explicitly quantify sky exposure and determine whether it directly affects seedling survival.

Some moss seedbeds can physically inhibit the growth of conifer seedlings through smothering (Hornberg et al. 1997), though mortality through seedbed overgrowth was very low on *Pleurozium* and *Cladina* treatments (<5% over 2 years). In the Mealy Mountains, *Pleurozium* growth is likely comparable to the yearly shoot growth rates of the feathermoss *Hylocomium splendens,* which averaged ~10 mm per year; this falls at the low end of the growth range for *H. splendens* (Koncz, unpublished). In the boreal forest, *Pleurozium* shoots grow at a rate of 10-30 mm per season (Longton and Greene 1969, Havas and Kubin 1983); like *H. splendens*, this growth rate is almost certainly smaller in the forest-tundra transition zone due to the shorter growing season. Black spruce seedlings growing on *Pleurozium* grew ~8.0 mm on average in the first growing season, and ~6.0 mm in the second, making overgrowth by *Pleurozium* slow or unlikely to occur. *Cladina* seedbeds are more difficult to assess in terms of yearly height increase, since these lichens lack the clear growth intervals visible in mosses such as *H. splendens*; *Cladina* growth rate is assessed in terms of ($g\ g^{-1}\ year^{-1}$). There are no estimates of *Cladina* yearly growth rate for the Mealy Mountains forest-tundra transition zone, but research suggests that *Cladina* in subarctic heath habitats above the standing treeline have a comparable growth rate to subarctic ericaceous plants (den Herder et al. 2003). In

subarctic Quebec, *Cladina* at the periphery of caribou-disturbed sites grow approximately 5.6 mm/year (Boudreau and Payette 2004). These relatively low growth rates, coupled with the low seedling mortality from seedbed overgrowth (<5%), suggest that the moss and lichen seedbeds are not likely to inhibit black spruce survival through smothering.

Seed and seedling herbivory

Seedbeds may facilitate black spruce survival by sheltering seeds and seedlings from predators. Slugs were predicted to be the principal predators of seedlings planted in the Mealy Mountains forest-tundra boundary zone after prior observance of slug damage to planted seedlings (Munier 2006). Herbivory by slugs was indeed the major cause of seedling mortality during the first growing season, accounting for more than 90% of all herbivory. After the first summer (2007), seedling herbivory underwent a sharp decline into the second growing season. In 2008, there were only 7 new herbivory events that could be attributed to slugs, compared to 76 events in 2007; for 2008, this ranks herbivory behind overwinter mortality (45 events), desiccation (35 events) and unknown causes (9 events) in terms of seedling mortality factors. Seedling age is known to decrease the palatability of some plant species to *Deroceras spp.* (Hanley et al. 1995) and a comparison of slug-related seedling deaths in 2007 and 2008 suggests that black spruce palatability (and thus vulnerability to slugs) declined after the first growing season. Alternately, slug populations may have been lower in 2008, but no sampling data were collected in 2007 for comparison. Some *Deroceras* species are known to have disproportionately high grazing rates on rare species due to the need to seek a varied diet

(Cottam 1985), so *D. laeve* may be motivated to feed on new plant seedlings introduced to its habitat, such as black spruce. This may significantly affect any seedlings that may naturally recruit in the forest-tundra ecotone in the future.

Seedlings were differentially targeted across different seedbeds, supporting the prediction of facilitation. Herbivory from slugs varied among the seedbed treatments with seedling herbivory having greater odds of occurring on dig plots than on those with intact seedbeds. The lower probability of slug herbivory on intact seedbeds (both *Cladina* and *Pleurozium*) relative to digs suggests that the intact seedbeds actively shelter seedlings from slug predators during their first growing season. Though the experimental plots are on a smaller spatial scale, these results are consistent with the Côté et al. (2005) study of black spruce seedling herbivory in the boreal forest where slugs were a dominant seedling predator and invertebrate predation was highest in burned areas with a disturbed seedbed.

In both the first growing season (2007) and the two-year study period (2007-2008), seedling herbivory attributed to slugs was almost equally likely to occur on plots scattered with organic slug pesticide than plots with no pesticide treatment, and there was no significant seedbed-pesticide interaction effects. This suggests that the pesticide pellets, while possibly effective at killing slugs later, did not prevent them from feeding on black spruce seedlings while they were in the study area. Slug pesticides containing ferric phosphate have been used effectively to reduce *Deroceras reticulatum* herbivory in agricultural field trials (Speiser and Kisler 2002), but have never been applied to *D. laeve*

in monitored trials. The pellets must be ingested to kill the slugs, and since no dead or dying slugs were ever found in proximity to the pesticide-treated plots, *D. laeve* may not have ingested the pellets; pellets were often still present on plots during the periodic pesticide re-application, so this is a possible explanation for the equal likelihood of slug herbivory under +/- pesticide treatments. Alternatively, slugs may have grazed seedlings, ingested the pesticide pellets, and then retreated under the groundcover to die.

The pesticide treatment also had a negative effect on seedling root development, as the odds of a seedling having an underdeveloped root system were almost twice as high as those on untreated seedbeds. This had a negative impact on seedling survival, so in future, this type of pesticide treatment should be avoided in black spruce seedling studies.

Granivory trends were similar to seedling herbivory, with granivory events having equal odds of occurring on digs and *Cladina* and relatively lowest odds of occurring on *Pleurozium* treatments. These results correspond to Côté et al.'s (2005) findings in the boreal forest habitat, where predation of post-dispersal black spruce seed was lowest in *Pleurozium*-spruce forests, followed by *Cladina* and was highest in non-vegetated burned sites. The increased likelihood of predation on pesticide-treated plots suggests either that, as in the seedling herbivory study, the pesticide is ineffective against slugs, or that non-slugs granivores are feeding on black spruce seed. Further research is needed to determine the common granivores in the Mealy Mountains forest-tundra ecotone.

2.4.2 Implications of seedbed facilitation for black spruce recruitment and tree expansion

Black spruce was able to germinate, root in the seedbed, and survive their two growing seasons above the treeline in the Mealy Mountains forest-tundra ecotone, which was consistent with Munier's (2006) findings of introduced seedlings establishing successfully in the alpine tundra; however further research needs to be done on the impact on seedlings once they grow beyond the sheltering effects of the surrounding seedbed since other vegetation interactions may increase in importance, such as potential facilitation by shrubs (Cranston 2009). The potential for *Pleurozium* facilitation of seed emergence and early establishment above the standing treeline exists, if viable seeds are being produced within the forest-tundra boundary, or transported there from the lower-altitude boreal habitat. Lack of viable seed is an important factor limiting plant establishment in alpine habitats and the forest-tundra ecotone (eg. Lindgren et al. 2007); currently in the Mealy Mountains, black spruce seed production at the treeline is a bottleneck to establishment (Jameson, in progress).

Moss and lichen seedbeds represent a significant proportion of high-latitude plant diversity and biomass and are important for habitat structure and nutrient cycling in these habitats. In the Mealy Mountains forest-tundra ecotone, mosses compose an average 20% of the groundcover, while lichen seedbeds account for an average of 15%; shrubs, vascular plants, and other groundcovers account for the remaining area. However, there are few studies that examine the impact of shifting climate regimes on these under-

canopy communities (Nilsson and Wardle 2005, Jagerbrand et al. 2006). Changes in soil moisture, canopy cover and community composition could cause a shift in dominant seedbed. For example, an increase in soil moisture (through increased precipitation/snowmelt, a possible future scenario for Labrador) can cause a shift from a dominant *Pleurozium* seedbed to *Sphagnum* (Gilliam and Roberts 2003), a genus that improves black spruce germination but tends to smother seedlings and may have an allelopathic effect on seedlings (Verhoeven and Toth 1995, Hornberg et al. 1997). Warming temperatures and increased nutrient availability could favour an increase in vascular plant biomass over lichen groundcovers (Cornelissen et al. 2001).

Ground-layer species can also be strongly affected by changes in the light availability caused by changing canopy composition and distribution, such as that caused by advancing shrubs (Van Wijk et al. 2003, Sturm et al. 2005). *Pleurozium* seedbeds cannot grow in unshaded habitats (Bisbee et al. 2001), so an advancing shrub canopy may facilitate the growth of this groundcover; in the Mealy Mountains *Pleurozium* is almost always found in proximity to a shrub or krummholz canopy. Shrubs were not shown to facilitate early black spruce recruitment in the Mealy Mountains (Cranston 2009), so moss seedbeds may act as critical early microhabitats for black spruce. A shift towards a more facilitative seedbed could increase the recruitment of black spruce in the treeline ecotone and promote tree expansion upslope, or vice versa; thus it is important to understand how seedbeds are likely to respond to climate change in the Mealy Mountains. A shift to a climate favouring the dominance of feathermoss seedbeds may

act to facilitate the advance of a black spruce treeline by providing optimal microhabitat for emergence, growth and survival.

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3.0 Conclusions

The pattern of black spruce seedling recruitment in the Mealy Mountains forest-tundra ecotone is characterized by best growth and survival on *Pleurozium* seedbeds and lowest on bare soil digs. This represents a completely different recruitment pattern than seedlings in the boreal forest: in this habitat, black spruce generally show best survival and recruitment on mineral soils in the absence of competitive *Pleurozium* and *Cladina* seedbeds (e.g. Prévost 1997, Charron and Greene 2002, Hébert et al. 2006). Since the opposite recruitment trend is evident in the alpine forest-tundra ecotone, this suggests a shift from the seedling-*Pleurozium* competition characteristic of the boreal habitat to facilitation, as predicted by the Stress Gradient Hypothesis (SGH) (Lortie and Callaway 2006). This facilitative black spruce-*Pleurozium* interaction could promote tree expansion into the alpine tundra since *Pleurozium* is extremely common in the forest-tundra ecotone and thus the likelihood of black spruce seeds landing and germinating within this seedbed are high.

Black spruce is an important species in the forest tundra ecotone because it is the most abundant conifer and is the largest seed contributor in the ecosystem (Jameson, unpublished). Black spruce seeds germinated, and planted seedlings survived two seasons in the forest-tundra ecotone. Thus, the potential for *Pleurozium* facilitation of seed emergence and early establishment above the standing treeline exists if viable seed is present. Lack of viable seed often limits plant establishment at the treeline (eg. Lindgren et al. 2007) and is thought to be a bottleneck to current black spruce

establishment in the Mealy Mountains, though seed availability/viability may increase under the influence of climate warming (Jameson, in progress).

The limitations of this project were primarily study duration and spatial scale. After two growing seasons, black spruce seedlings have just emerged above the surface of the vegetated seedbeds. Close to the ground, seedlings benefit from growing in a microclimate that may be dissimilar to the free atmosphere (Holtmeier and Broll 2005). Once seedlings emerge from the boundary layer above the protective *Pleurozium* seedbed, other vegetation interactions may increase in importance, such as potential facilitation by shrubs (Cranston 2009). The study was conducted on a small spatial scale, both for logistical reasons and to reduce intra-site variation. It is unknown whether *Pleurozium* seedbeds elsewhere in the Mealy Mountains forest-tundra ecotone, or in other alpine treelines, would demonstrate the same facilitative interactions with black spruce seedlings.

Future research could amend these limitations by continuing the study past the original two-year duration to examine the impacts of seedbed on 2+ year-seedlings, and expanding the study area. Munier (2006) was the first to demonstrate the potential for seedling establishment above the current treeline and established experimental seedling plots along an altitudinal gradient (forest, forest-tundra, alpine tundra, etc.). Using Munier's study as a model, experimental plots could be established along an altitudinal gradient within the valley to examine whether *Pleurozium* facilitative effects increase seedling growth/survival at higher elevations beyond the current range of conifers, within

the actual alpine tundra. Experimental plots could also be established on the same seedbeds at similar latitudes, elevations and slope aspects in the forest-tundra ecotone of other highland areas to determine whether the *Pleurozium*-black spruce facilitation observed in this study would be evident outside the Mealy Mountains.

Understanding the influence of biological interactions on conifer recruitment is critical for predicting how tree range will expand under the influence of warming climates. The advance of boreal conifers may seriously impact genetic diversity, species diversity and areal extant of locally rare alpine tundra (Munier 2006, Malanson et al. 2007), thus contributing to the changing face of the northern landscape.

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Appendix 1

Generalized linear models for statistical analyses

The majority of seedling data (seedling herbivory, mortality, root inhibition and seed predation) were analysed using binomial logistic regression models. Logistic regression was originally developed as a tool for epidemiological research, but has expanded into multiple fields, including ecology. It can be distinguished from linear regression by its binary outcome variable, where response can be differentiated into one of two possible outcomes (i.e. present/absent, alive/dead) (Hosmer and Lemeshow 2004). Parameter estimates generated by a logistic regression model can be transformed to calculate odds and odds ratios, which can then be used to observe the magnitude of a treatment effect: they compare the odds of an event (herbivory, mortality, etc) occurring on one treatment relative to another, reference (i.e., control) treatment.

Odds and odds ratios may be somewhat non-intuitive when compared to chance probabilities, but are relatively simple to understand. The parameter estimate for a logistic regression intercept can be transformed to generate the odds of a specific event occurring on a reference treatment (in the case of all analyses in this study, digs with no pesticide). If the calculated odds are greater than 1 for this treatment, the event is more likely to happen than not (greater than 50% chance of occurring); if the odds fall closer to 0, this denotes a more unlikely event (with odds= 0 being an impossible event). The odds ratios, which are generated by the transformation of the rest of the model's parameter estimates, denote the likelihood of the event occurring on each treatment *relative* to the

reference treatment. In this case, an odds ratio greater than 1 indicates an event is more likely to occur on the second treatment relative to the reference treatment, and an odds ratio less than 1 correspondingly indicates that the event is less likely to occur. Transformation of each parameter's Wald 95% confidence intervals gives the confidence limits of the odds and odds ratios. If the range of the odds ratio's confidence intervals does not cover 1, the probability of the effect being caused by chance is under 0.05 (Deeks 1996). Thus, by using logistic regression models, it is possible to observe the likelihood of an event on the reference treatment, determine whether it is more or less likely to occur on the other treatments, and demonstrate the magnitude of this difference in likelihood.

Appendix 2

Soil nutrient supply principal components analysis (PCA)

Seedbed nutrient availability may be one of the most important determinants of black spruce seedling recruitment (Lavoie et al. 2006); thus, in a cold, low-nutrient system like the forest-tundra ecotone, seedlings may benefit from establishing in a seedbed with a relatively high nutrient supply. *Pleurozium* was expected to have a greater nutrient concentration (particularly nitrogenous compounds) than *Cladina*, because *Cladina* seedbeds are characteristically nutrient-deficient (Bonan and Shugart 1989, Carleton 1990), and because *Pleurozium* may be associated with nitrogen-fixing cyanobacteria (DeLuca et al. 2002, see Appendix 6).

Analysis of the first two principal component axes explained 71% of the variation in soil nutrient supply rates (Table A2.1); PCA axis 1 primarily reflected calcium, magnesium and iron concentrations, as indicated by their negative (less than -0.495) component loadings, while PCA axis 2 mainly reflected positive iron and negative sulphur (0.607 and -0.569) loadings.

Table A2.1: Loadings for each component of nutrient/seedbed Principal Components Analysis; bolded numbers represent significant loadings (greater than 0.5 or less than -0.5)

	PC1	PC2
N	-0.193	-0.225
NO3	-0.162	-0.285
Ca	**-0.497**	-
Mg	**-0.554**	-0.105
K	-	0.227
P	-	-
Fe	**-0.499**	**0.607**
Zn	-0.190	-0.244
B	-	-
S	-0.218	**-0.569**
Al	-0.174	-0.223
Standard deviation	0.2206216	0.1654678
Proportion of Variance	0.4573725	0.2572770
Cumulative Proportion	0.4573725	**0.7146495**

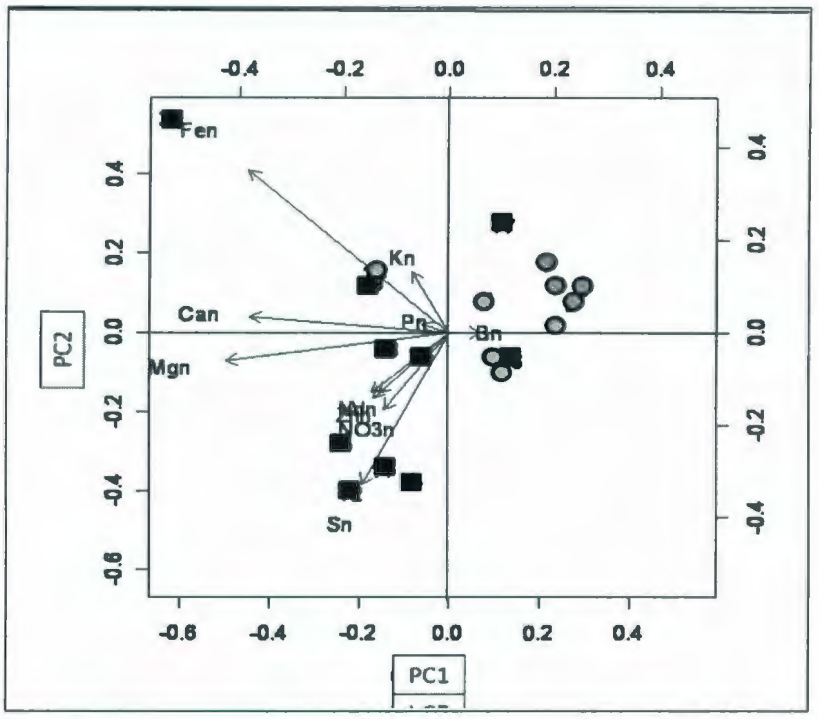

Figure A2.1: Biplot showing relationships between PC1 and PC2 Euclidean-normalized nutrient availability (N_n= total nitrogen, $NO3_n$=nitrate, Ca_n=calcium, Mg_n=magnesium, K_n=potassium, P_n=phosphorous, Fe_n=iron, Zn_n=zinc, B_n=boron, S_n=sulfur, Al_n=aluminum) and seedbed (grey circles=*Cladina*, black squares=*Pleurozium*); tight *Cladina* cluster indicates a common pattern of characteristic nutrient deficiency in the sampled *Cladina* seedbeds (poor in most available nutrients); dispersed (non-grouped) *Pleurozium* points indicate that while nutrient availability is higher on *Pleurozium* (points positively associated with red nutrient arrows), there is no common nutrient pattern

Black spruce growth (% height increase) was significantly greater on *Pleurozium* seedbeds relative to *Cladina*. Greater nutrient availability in the moss seedbed may have contributed to this observed result. For further detail on effects of seedbed nutrient availability on black spruce growth and survival, see Section 2.4.1.

Appendix 3

Survival-weighted seedling % height increase (2007-2008)

Average height increase was greatest on *Pleurozium*+ pesticide and *Pleurozium* treatments in the 2007 and 2008 growing seasons. After summer 2007, survival-weighted % height increase was greatest on *Pleurozium*+ (31.5%), followed by Dig+ (24.8%), *Pleurozium*- (23.4%), *Cladina*- (20%), *Cladina*+ (18.2%) and Dig- (16.3%). After 2007, slug pesticide had no significant effect on seedling growth, so data were pooled. After the first winter, in June 2008, survival-weighted % height increase was greatest on *Pleurozium* (31.1%), followed by *Cladina* (20.0%) and Digs (17.3%). After the second growing season in September 2008, survival-weighted % height increase was again greatest on *Pleurozium* (30.9%), followed by *Cladina* (21.7%) and Digs (20.5%).

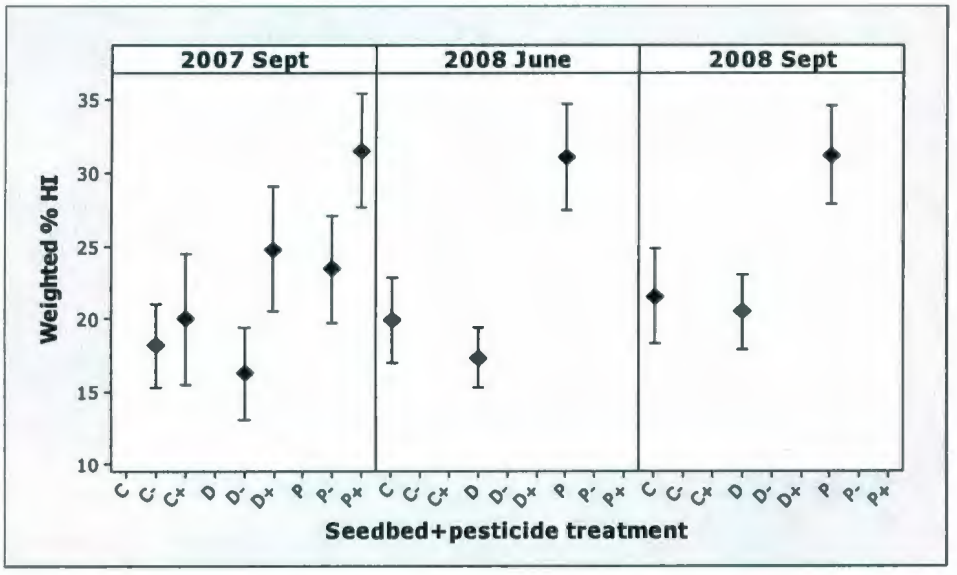

Figure A3.1: Seedling survival-weighted percent height increases (with 95% CI) of seedlings on Dig +/- (D+/-), Cladina +/- (C+/-) and Pleurozium +/- (P+/-) (2007-2008); 2008 data are pooled due to no significant difference between pesticide treatments in 2008

Appendix 4
Slug population sampling

Deroceras laeve, the local slug species, is a known predator of black spruce seedlings (Munier 2006), and since slug herbivory is a controlled variable, an estimate of population size in the study area was needed. A passive sampling study was carried out during the 2008 field season; eighteen (18) flat 10 cm^2 plastic slug shelters were haphazardly deployed across the study area, with six (6) in both the pesticide and non-pesticide study areas, and a further six in a nearby *Sphagnum* and *Betula*-dominated habitat. The study was carried out on a 3-day interval (3 days on, 3 days off), with three repetitions. Shelters were examined at 6 AM each morning of the study to observe the number of slugs collected on the under-surface; temperature (°C) and relative humidity (%) at ground level were measured and recorded to determine under what conditions slugs are active.

Figure A4.1: *Deroceras laeve* on the underside of a passive slug shelter (photo credit B. Cranston 2008)

Despite the intense sampling effort, only 4 slugs were trapped during the 2008 growing season. However, slugs had a substantial impact on seed and seedlings survival in the treeline study area; adults and eggs were found on the seedling peat pellets, and the feeding patterns left on the seedling needles and rasped seeds are distinctive to slugs. The low number of captured slugs may be due to several reasons. First, the traps themselves were simply passive shelters, with no bait to attract the slugs; given the complex physical structure of the groundcover (dense moist seedbeds, deciduous vascular litter, etc.), there was likely an abundance of suitable shelter available. Secondly, the size of the area covered by the traps (10x10 cm x 18 shelters) was miniscule compared to the size of the study areas. Therefore, the four (4) slugs recorded should not be taken as an indication of a small slug population.

Appendix 5

Black spruce versus white spruce emergence

Thirty seeds of both black and white spruce were sown and marked on 10 *Cladina* plots, 10 *Pleurozium* plots and 14 bear dig plots from the seedling establishment study on June 23/08, for a total of 1020 seeds of each species. The seeds were monitored every day and emergence was quantified for each seedbed type. A Poisson regression model was used for seed emergence, with count of emerged seeds as the response variable, and seedbed and seed species as the explanatory variables.

During the 2008 growing season, emergence was significantly different across seedbed (Chi-square=376.34, p=<0.0001) and seed species (Chi-square=9.01, p=0.0027), with significant interactive effects (Chi-square=28.15, p=<0.0001) (Table 2.3.1). Black spruce emergence was highest on *Pleurozium* (N emergents=19, 6.3%), followed by dig (N emergents=16, 5.3%) and was lowest on *Cladina* (N emergents=1, 0.33%). White spruce emergence was highest on digs (N emergents=22, 7.3%), followed by *Pleurozium* (N emergents=14, 4.7%) and was lowest on *Cladina* (N emergents=3, 1.0%). Seed emergence was earliest on dig treatments (wS emergence between Jul 8-Sept 13, bS July 12-Sept 13), followed by *Pleurozium* (wS emergence between July 13-Sept 13, bS July 13-Sept 13), with latest emergence on *Cladina* (single bS emergence on July 17, wS July 17-Sept 13).

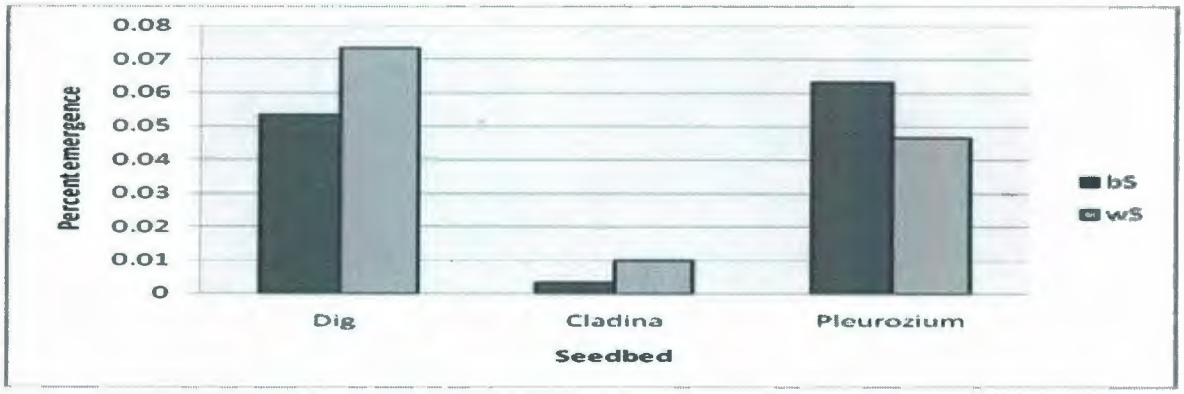

Figure A5.1: Proportion of black spruce (bS) and white spruce (wS) emergence across dig, *Cladina* and *Pleurozium* seedbed treatments (2008)

Table A5.1: Incidence of black spruce (bS) seed emergence on Dig (incidence =16) and incidence ratios of relative emergence likelihood by seedbed and seed species (2008); ratios >1 = higher emergence likelihood, ratio <1 = lower emergence likelihood

	Incidence Ratio		**IR Confidence Intervals**
***Cladina* v Dig, bS**	0.0625	0.0332	0.118
***Pleurozium* v Dig, bS**	1.188	0.979	1.441
wS v bS	1.375	1.158	1.633
wS v bS, on *Cladina*	3.000	1.210	7.439
wS v bS, on *Pleurozium*	0.737	0.470	1.156

During the 2008 growing season, white spruce had a higher overall incidence, or probability of emergence relative to black spruce (OR=1.375), on all seedbeds except for *Pleurozium*, where the incidence of white spruce emergence relative to black were IR=0.737.

White spruce is present, but occurs at a lower abundance than black spruce in the Mealy Mountains treeline ecotone. The odds of black and white spruce germination on Digs and *Pleurozium* were relatively similar, while on *Cladina*, the odds were 3x higher

for white spruce (OR=3.0). However, seed emergence on *Cladina* was so low (white spruce: 0.01% vs. black spruce: 0.003%; Figure A5.1) that this odds ratio is deceptively high. Since white spruce had slightly greater overall odds of germination relative to black (OR=1.375), it may recruit at a comparable rate to black spruce, although further research is needed to determine the influences of seedbed, predators and other local ecological factors on white spruce seedlings.

Appendix 6

Pleurozium-Nostoc association

Pleurozium schreberi is a common shade-tolerant feathermoss (Bisbee et al. 2001); in the boreal forest, it efficiently intercepts and sequesters nutrients, usually leading to a nutrient deficit within the community (Zackrisson et al. 1997, Wardle et al. 1997, Zackrisson et al. 1999, DeLuca et al. 2002). In boreal studies, this species is generally considered to be a suboptimal seedbed for black spruce (Prévost 1997, Charron and Greene 2002), with few exceptions (Lavoie et al. 2006). However, *Pleurozium* is also capable of nutrient mobilisation when associated with a nitrogen-fixing cyanobacterium, *Nostoc sphaericum*; this association has been estimated to contribute a significant proportion of the organic nitrogen that is fixed yearly in the boreal forest (DeLuca et al. 2002, Houle et al. 2006, DeLuca et al. 2007). The association between *Pleurozium schreberi* and *Nostoc sphaericum* was first identified by DeLuca et al. (2002) in samples collected from boreal forest sites in northern Sweden, Norway and Finland, from which they estimated an average N-fixation rate of 1.6 kg N per hectare per year. This association has also been detected and studied in the boreal forest of northern Quebec (Houle et al. 2006). To date, it has not been detected in Labrador.

Ten moss samples were taken from krummholz, dwarf birch and open-canopy forest habitats (30 *Pleurozium* samples total) in mid-July 2008 to test whether this cyanobacterial association is present in the Mealy Mountains. These samples were observed at 200-400x using an ultraviolet-fluorescent microscope under a green filter

following the protocol in DeLuca et al. (2002); if *Nostoc* is present, it will be visible as coiled red cells in the incurve of the moss leaves.

Moss samples taken from along a forest-tundra boundary habitat gradient (closed-canopy forest, krummholz, and a dwarf birch stand) in the Mealy Mountains during July 2008 showed no physical evidence of cyanobacterial associations. However, *Pleurozium* samples previously taken during July and August in other boreal locations in Sweden also have low relatively or undetectable nitrogen-fixation rates (Rosen and Lindberg 1980); this can be explained by the overlap of the sampling season with a period of potential feathermoss dormancy. In addition to this, high nitrogen fixation rates have been observed in *Pleurozium* in the boreal forests of Alaska, with no detectable cyanobacteria (DeLuca et al. 2002). Further sampling was planned for September 2008 to investigate whether a cyanobacterial association would be observable in *Pleurozium* sampled during a potentially more active period for feathermoss. However, sampling could not be carried out due to poor weather conditions in the field.

The results of the seedbed nutrient PCA (Appendix 2) characterize *Pleurozium* seedbeds as more nutrient-rich than *Cladina*, and the elevated nitrogen compounds in moss suggest there may be potential for this association but more study is needed to determine the dynamics of the *Nostoc/Pleurozium* association in the Mealy Mountains forest-tundra ecotone.

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